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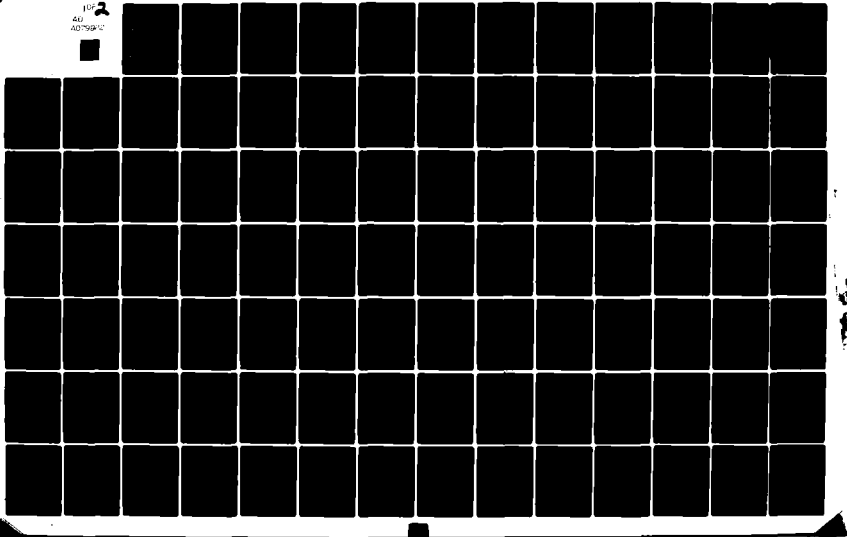

ENVIRONMENTAL EFFECTS ON THE ADHESION OF *Enteromorpha clathrata*

John Campbell III

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NAVY DEPARTMENT

NAVAL SEA SYSTEMS COMMAND

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The green alga Enteromorpha has been implicated as the predominant organism in the fouling of present-day oil tankers. This alga adheres to the hulls of ships by means of an adhesive matrix composed of carbohydrate and protein. The adhesiveness of this matrix has been found to be influenced by such environmental factors as light, darkness, temperature, and calcium. Light and darkness are correlated with degradative enzymes, while calcium is implicated in causing a hardening of the adhesive matrix. The light effect

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20. ABSTRACT (Continued)

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ABSTRACT

The green alga Enteromorpha has been implicated as the predominant organism in the fouling of present-day oil tankers (18,24). This alga adheres to the hulls of ships by means of an adhesive matrix composed of carbohydrate and protein. The adhesiveness of this matrix has been found to be influenced by such environmental factors as light, darkness, temperature, and calcium. Light and darkness are correlated with degradative enzymes while calcium is implicated in causing a hardening of the adhesive matrix. The light effect appears to initiate a phytochrome response which regulates those degradative enzymes responsible for decreasing adhesion. Temperature is indirectly related to adhesion by affecting growth.

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ABBREVIATIONS

DCMU	3-(3,4-Dichloropheney)-1,1-dimethyl urea
HOEDTA	Chel DM Acid
Na_2EDTA	Disodium Ethylenediamine-tetraacetate

ACKNOWLEDGMENTS

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I. INTRODUCTION

Biodeterioration is any undesirable change in the properties of a material caused by the vital activities of organisms (24). As such, it is a familiar and very wide-spread phenomenon which affects most organic material and some inorganic material. Its consequences are expensive to man. An estimate of 10^9 dollars per year was suggested as a lower limit of its cost at the Proceedings of the Fi International Biodeterioration Symposium in 1968 (24). One kind of biodeterioration is known as marine fouling.

Marine fouling is the result of settlement and growth of plants and animals on surfaces submerged in the sea (18). The detrimental effects of fouling are well known and of wide range. The following are some of the results of marine fouling: increased resistance to flow of ships' hulls resulting in a reduction in speed, decreased water flow through pipes, plugged orifices, added weight, interference with moving parts (2), loss of efficiency of sonar equipment (16), interference with environmental sensors, destructive actions upon coatings, and accelerated corrosion of metals (2).

The problems of marine fouling have plagued mankind for as long as the oceans have been used. To operators of large ocean-going vessels, particularly tankers, marine fouling presents major economic problems. For example, increased frictional resistance of the hull due to fouling leads to an increase in fuel consumption if the ship's scheduled speed is to be maintained. Inevitably, fouling leads to a loss in speed. The cost to a tank owner due to loss of speed and increased usage of fuel for a 6,000-ton ship can be $33,000 per year. Removal of the growth on the hull necessitates dry docking and possibly long periods out of

service. The total cost of a week in dry dock for an 11,000-ton tanker can be as much as $50,000 (18). These are 1971 costs and the size of tankers mentioned are small. The costs for the larger tankers having an approximate size of 122,000 tons would certainly exceed the previously mentioned costs.

The first written records of shipbottom treatment date from the fifth century B.C. (2). Historically, the development of methods to prevent fouling are divided into three parts: 1) the discovery of copper sheathing as an effective antifouling surface; 2) the introduction of metallic hulls resulting in the invalidation of metallic copper due to its promotion of galvanic corrosion; 3) the development of antifouling paints which to date remain the major antifouling method (2). During the last four decades, many other antifouling methods have been proposed and tested. These have varied from isotope introduction of metal or coatings, electrical systems, ultrasonics, use of chlorine (24), and periodic underwater hull cleaning. Still, no effective method of any appreciable longevity has been demonstrated. Toxic paint coatings remain the predominant preventative technique. The maximum theoretical life which can be attained by a toxic coating is on the order of three to five years, but the practical life on a shipboard system is generally from one to one and one-half years in duration (16).

Present-day oil tankers are generally free from animal fouling organisms. The reason for this appears to lie in the mode of tanker operation; short stays in port for taking on or discharge of oil or gas, routings which take the vessel from tropical waters to temperate waters in a short time, and the high speeds attainable by these large tankers. This wide range of environmental conditions, while largely excluding

animal fouling, is highly favorable to algae (8). Enteromorpha sp. and Ectocarpus sp. are the predominant algae found on the hulls of large tankers (1,9). Of the two, Enteromorpha, (the "green" weed) is the more prevalent for the following reasons: Enteromorpha can survive wide extremes of temperature and salinity; it requires only inorganic nutrients and light for growth; it can adhere strongly to surfaces; and it is not hindered in its growth by the speeds attained by large tankers, but is actually stimulated (18,24).

The phenomenon of ship-fouling and attempts to cope with it are common problems for ship owners. As a branch of scientific inquiry, however, the study of ship-fouling is still in its infancy. Since ship-fouling is a biological phenomenon, it is necessary to have a fundamental understanding of the biotic factors involved in order to develop and predict the degree, composition, and method of control most effective in preventing fouling. Much of the present work on prevention of ship-fouling is aimed toward understanding the nature of the organisms involved and the chemical characterization of the adhesive or cementing compounds these organisms produce.

The most significant work to date on the culture, morphology, cytology, and reproduction of Enteromorpha has been done by Ramanathan (15) and Kapraun (11). Enteromorpha is a green alga (Chlorophyta) and belongs to the order Ulvales. Together with Ulva, they are the most conspicuous of the green algae in the oceans. They occur in all seas, and often are prevalent in brackish or polluted areas, or in salt marshes. Enteromorpha is probably the most common of all the benthic green algae (6). This alga consists of a tubular thalli which is monostromatic and possesses a system of branching of various orders. The

alga is attached by a rhizoidal outgrowth (holdfast) at the base (Figure 1). The cells of the thallus are rectangular and in distinct longitudinal series. There is a single plate-like chloroplast, with one to three pyrenoids. The holdfast is composed of a number of closely-packed cells forming a parenchymatous disc-like expansion.

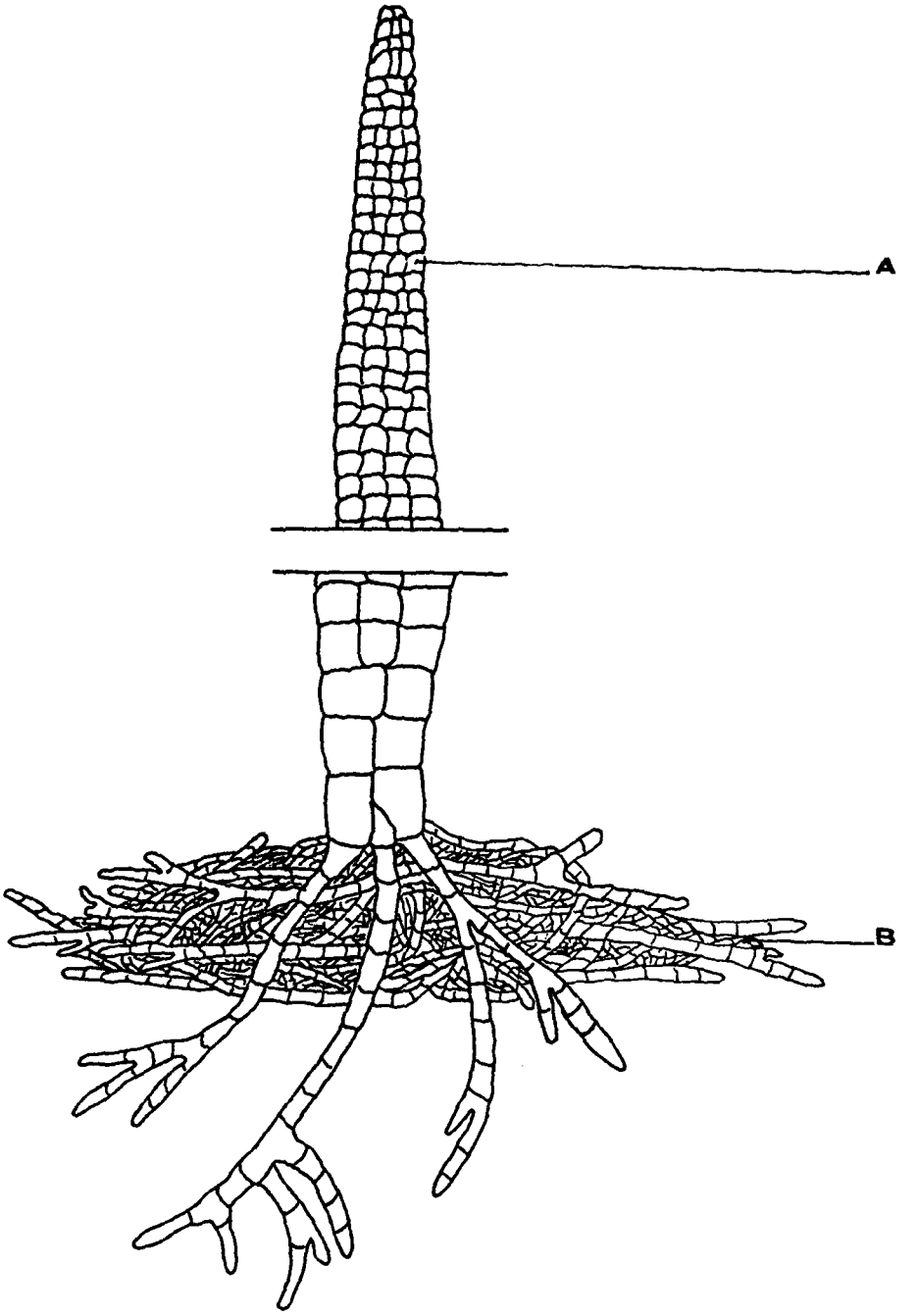
Enteromorpha usually has an alternation of identical gametophyte and sporophyte generations. The sporophyte may produce four, eight, or sixteen quadriflagellate zoospores in any cell but the holdfast. If a sexual generation occurs, biflagellate gametes are formed and liberated the same as zoospores. Fertilization is isogamous, anisogamous, and there can also be parthenogenetic germination of gametes. Germlings from zoospores, zygotes, and parthenogenetic gametes differentiate into a thalli cell and a rhizoidal cell after the first cellular division. All cells which originate from the rhizoidal cell contribute to the holdfast attachment system while cells which developed from the thalli cell contribute to the erect, photosynthetic portion of the plant. Enteromorpha can also develop through regeneration.

Evans and Christie studied the fine-structure and biochemistry of swimming and settled zoospores of Enteromorpha intestinalis in an attempt to elucidate the mechanism of attachment (9). These researchers looked at the deposition and chemical composition of various organelles in the zoospores that might play a role in attachment. They observed two types of vesicles that are thought to play a role in adhesion. The first are small vesicles (450-650 Å) which are superficially present at the top end of the apical dome. This location, coupled with the fact that they disappear early during settlement, suggests their involvement in the attachment process. Their small size and lack of abundance

Figure 1

Enteromorpha clathrata

Drawing of Enteromorpha clathrata with thallus (A) and holdfast (B) cellular organizations shown (15).


A
B

suggests that their content is not the sole or even major adhesive material. The second are large vesicles (1550-3000 Å) which contain a very electron-dense material. These vesicles cease to be synthesized and are totally absent one hour after settlement. Callow and Evans later showed that carbohydrate material is present in these vesicles and in the extracellular adhesive (3). Through electron microscopic autoradiography following L-leucine uptake, they showed that protein is also part of these vesicles (4). Through further work, they were able to work out a possible scheme for the synthesis of the adhesive material. The carbohydrate component of the adhesive material is polymerized in the Golgi cisternae after detachment from the Golgi apparatus, and the protein moiety is transported to the Golgi from the adjacent rough endoplasmic reticulum.

With the use of α-amylase and trypsin, Christie, Evans and Shaw verified the chemical nature of the adhesive material as being a glycoprotein substance (5). They also observed a decrease in the ability of these enzymes to affect attachment with time. This seemed to indicate a chemical change or hardening process taking place in the adhesive material.

These series of investigations deal only with the initial attachment by zoospores of Enteromorpha. The holdfast structure of this alga is composed of a large number of cells. All of the cells that lie adjacent to the surface of attachment are responsible for the attachment of the entire alga. Thus, we conclude that the zoospores are only responsible for the initial attachment, while the cells of the holdfast region are responsible for the major attachment of the alga.

The long range goal of this research is to develop an effective method of preventing ship-fouling by Enteromorpha. In order to accomplish this task, several lines of investigations must be undertaken. These investigations include: 1) the study of attachment under different environmental conditions; 2) the chemical and structural characterization of the adhesive compound; and 3) the biosynthetic process involved in the production of the adhesive compound. The specific goal of this thesis is to investigate the various environmental factors that affect attachment of the holdfast region.

II. MATERIALS AND METHODS

A. Organisms, Media and Growth

Enteromorpha intestinalis LB739, Enteromorpha clathrata LB1847 #Ea-2 male and LB1848 #Ea-8 female, Enteromorpha prolifera LB1849 #RP-D-1 female and LB1850 #RP-D-2 male, and Enteromorpha lingulate LB1855 #L- male and LB1856 #L+ female were obtained from the Indiana Culture Collection, Department of Botany, Indiana University, Bloomington, Indiana. Previous work in our laboratory (unpublished) showed the optimum grown and optimum adhesion to glass was by Enteromorpha clathrata LB1847 #Ea-2 male in medium A. Thus, this work deals only with Enteromorpha clathrata LB1847 #Ea-2 male.

Axenic cultures of Enteromorpha are grown, unless otherwise noted, in 60 ml wide-mouth Florence flasks using an artificial seawater medium designated medium A (see Table 1). All cultures were maintained in an environmental incubator set at a temperature of 20°C with illumination provided by ten (overhead) F72T12-CW-1500 fluorescent lights. The continuous incident light intensity was 4.37×10^{-9} Einsteins sec^{-1}.

All cultures were inoculated from a stock of homogenized cells prepared by scraping the algae from a stock culture and grinding them up in a Ten Broeck tissue grinder. The stock of homogenized cells was adjusted (by adding medium A) so that 1 ml of cells in 20 ml of medium A gave an OD_{550} of 0.2. One ml of the homogenized stock was inoculated into each 60 ml flask containing 20 ml of medium A. The ground up cells developed through regeneration into the mature alga. Each set of experiments was inoculated from the same stock of homogenized cells. All cultures were incubated for two weeks unless otherwise noted. This

Table 1

Culture Medium A[1]

Composition of culture media for Enteromorpha:

Compound	g/l
Na_2EDTA	0.03
NaCl	18.0
$MgSO_4 7H_2O$	5.0
KCl	0.6
$CaCl_2 2H_2O$	0.37
$NaNO_3$	1.0
KH_2PO_4	0.05
Trizma base[2]	1.0

Trace element composition:

Compound	mg/l
$FeCl_3 6H_2O$	3.89
H_3BO_3	34.3
$MnCl_2 4H_2O$	4.3
$ZnCl_2$	0.315
MoO_3 (85%)	0.03
$CuSO_4 5H_2O$	0.003
$CoCl_2 6H_2O$	0.0122

[1]Derived from Provasoli et al. (13) modified by Van Baalen (23) and Stevens et al. (19).

[2]Trizma base is adjusted to pH 8.2 with conc. HCl.

time provided for an even confluent growth on the bottom of the flasks with very little thalli development. Cultures wrapped in aluminum foil were used for dark incubation experiments.

The quantitative estimation of growth was determined by dry cell weight. The algae were removed from the flasks either by the method for determining percentage of adhesion (see below) or by removing all the cells with a rubber policeman. The cells were collected over a tared No. 1 Whattman filter paper. They are then dried in an oven for 48 hours at 85°C. After weighing on an analytical balance, the dry weight of the algal cells was calculated.

B. Percentage of Adhesion Determination

Adhesion was measured using an apparatus modified from Christie, Evans and Shaw (5) which is shown in Figure 2. The cultures were hosed with water at a pressure of 12 psi. The cells that came off the glass were removed and their dry weight determined as previously described. These cells were considered to have been unadhered. The cells still attached to the glass were removed with the use of a rubber policeman and their dry cell weight determined. These cells were considered to have been adhered. The percentage of adhesion was then calculated using the following formula:

$$\frac{\text{weight of cells adhered}}{\text{total weight of cells}} \times 100\% = \% \text{ adhesion}$$

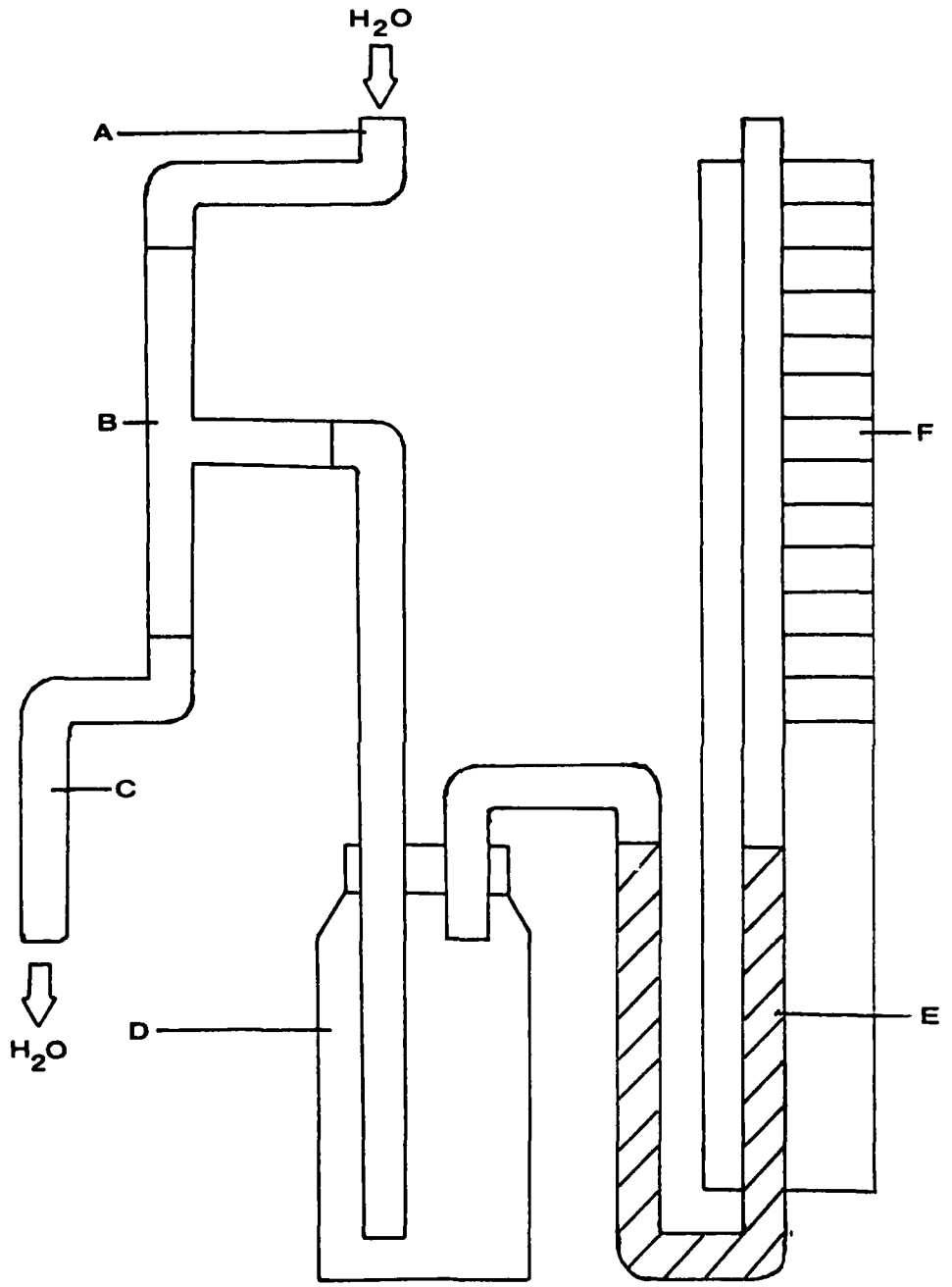
C. Determination of Light and Temperature Effects

To determine the effect of various light intensities and temperatures on the growth and adhesion of Enteromorpha, a light-temperature

Figure 2

Controlled Water-Pressure Apparatus Used for Assessing Adhesion of Enteromorpha

Tap water was connected to the water inlet (A). The water was diverted in the glass T (B) to the water outlet (C) and the water trap (D). Water entering the water trap caused air pressure to build up which forced the mercury (E) in the manometer (F) to rise. The manometer was in units of psi. The water from the outlet was used for hosing. Hosing was carried out on the culture twice for five seconds each.


H_2O
A
B
C
D
E
F
H_2O

gradient plate as described by Edwards and Van Baalen (7) was used. The gradient apparatus (Figure 3) concurrently provided results of light and temperature effects. The main part of the gradient apparatus was an aluminum plate 1-3/4 in. thick by 16 in. square. One side of the plate was heated and the other side was cooled by pumping water from two thermostatically controlled water baths through holes on each side of the plate. The flow of heat through the aluminum plate produced a linear temperature gradient that was perpendicular to the direction of water flow. A light intensity gradient at right angles to the temperature axis was provided by fluorescent or incandescent lights mounted on the front side of the plate. Thirty-six, 60 mm petri plates with 15 ml of medium A were inoculated with 1 ml of homogenized cells. The plates were placed on the aluminum plate giving six columns of increasing temperature and six rows of decreasing light intensity. Illumination was provided either by two 60W 115-125V lumiline (incandescent) lights or two F20T12/CW fluorescent lights. The arrangement of petri plates on the aluminum plate with temperature and light intensity indicated for the respective columns and rows is shown in Figures 4 and 5. Variation of light intensity with distance on the crossed-gradient plate for incandescent lights is shown in Figure 6A. Light intensity decreases in a curvilinear fashion with distance. A range from 12.8×10^{-10} Einsteins sec^{-1} to 1.3×10^{-10} Einsteins sec^{-1} was obtained. The sharp drop from 12.8×10^{-10} Einsteins sec^{-1} to 6.5×10^{-10} Einsteins sec^{-1} was due to the reflection of light off the tops of the petri plates becoming more pronounced as the distance was increased. Variation of temperature with distance under incandescent lights is shown in Figure 6B. Temperature increases linearly from Column 1 to Column 6.

Figure 3

Light and Temperature Cross-Gradient Apparatus

The 3/4-inch thick by 16-inch square aluminum plate (A) was supported in a metal and wood frame (B). The 36 petri plates were arranged in rows and columns on this plate. One side of the plate was heated and the other cooled by pumping water with a centrifugal water pump (C), from two thermostatically-controlled water baths through borings in each side of the plate from front to the back (D). The magnitude of the temperature gradient was controlled by adjusting the temperatures of the two baths. The two water baths were 12-inch by 12-inch cylindrical plexiglass jars (E) with lids. The warm water bath was heated by a 500W immersion heating coil (F) and a thermoregulator (G). The coldwater bath was cooled by a cooling coil (H) from a Constant Flow cooling unit (I). Light was provided either by two fluorescent or two incandescent lights (J) mounted on a board (K) and suspended above the front end of the plate. The lights could be moved forward or backward, and raised or lowered.

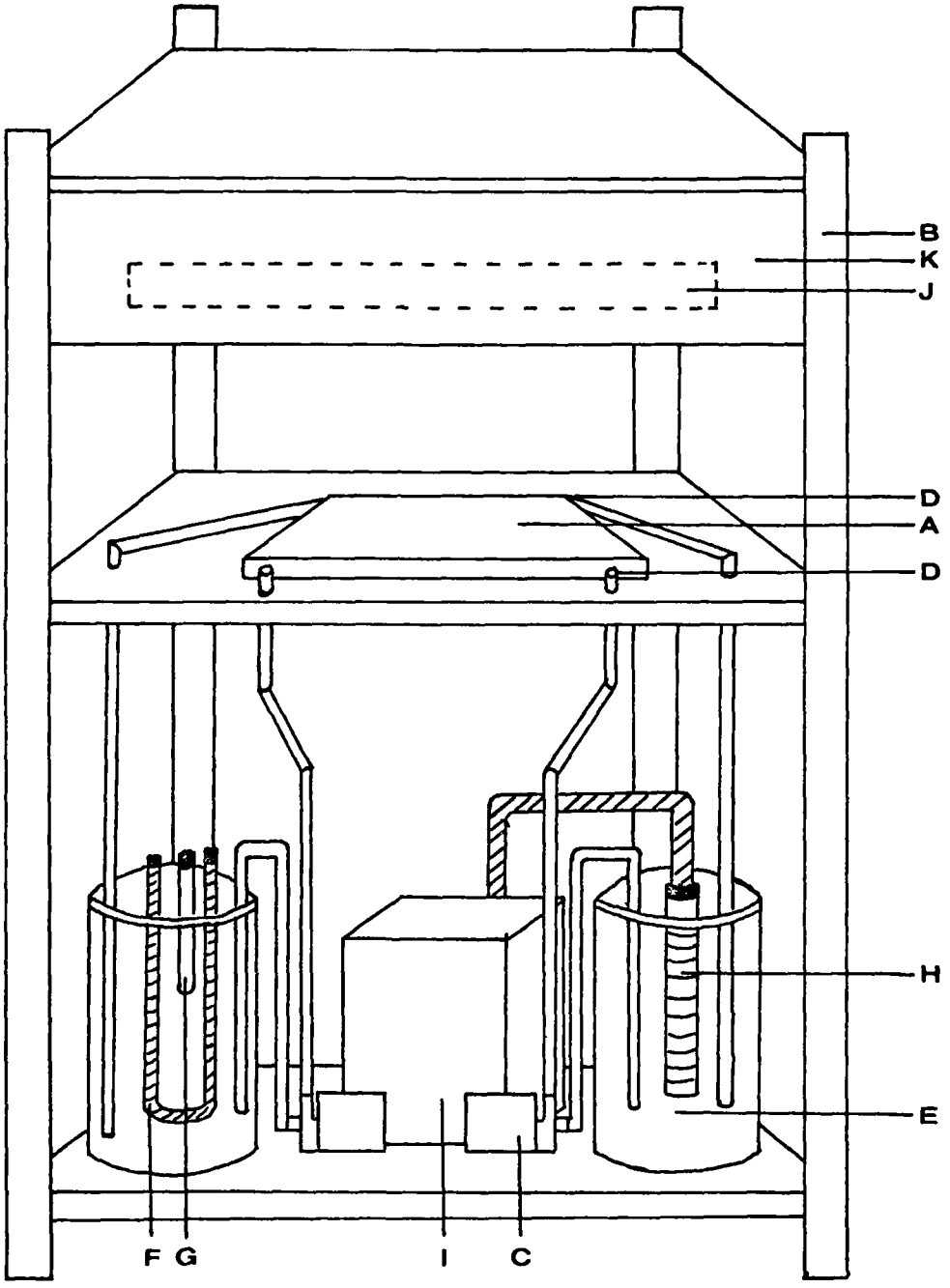

B
K
J
D
A
D
H
E
F
G
I
C

Figure 4

Light and Temperature Gradient Plate

The petri plates were arranged on the aluminum plate with temperature indicated by column and light intensity (incandescent) indicated by row. Temperature was measured in °C. Light intensity was measured in Einsteins sec^{-1} ($x10^{-10}$).

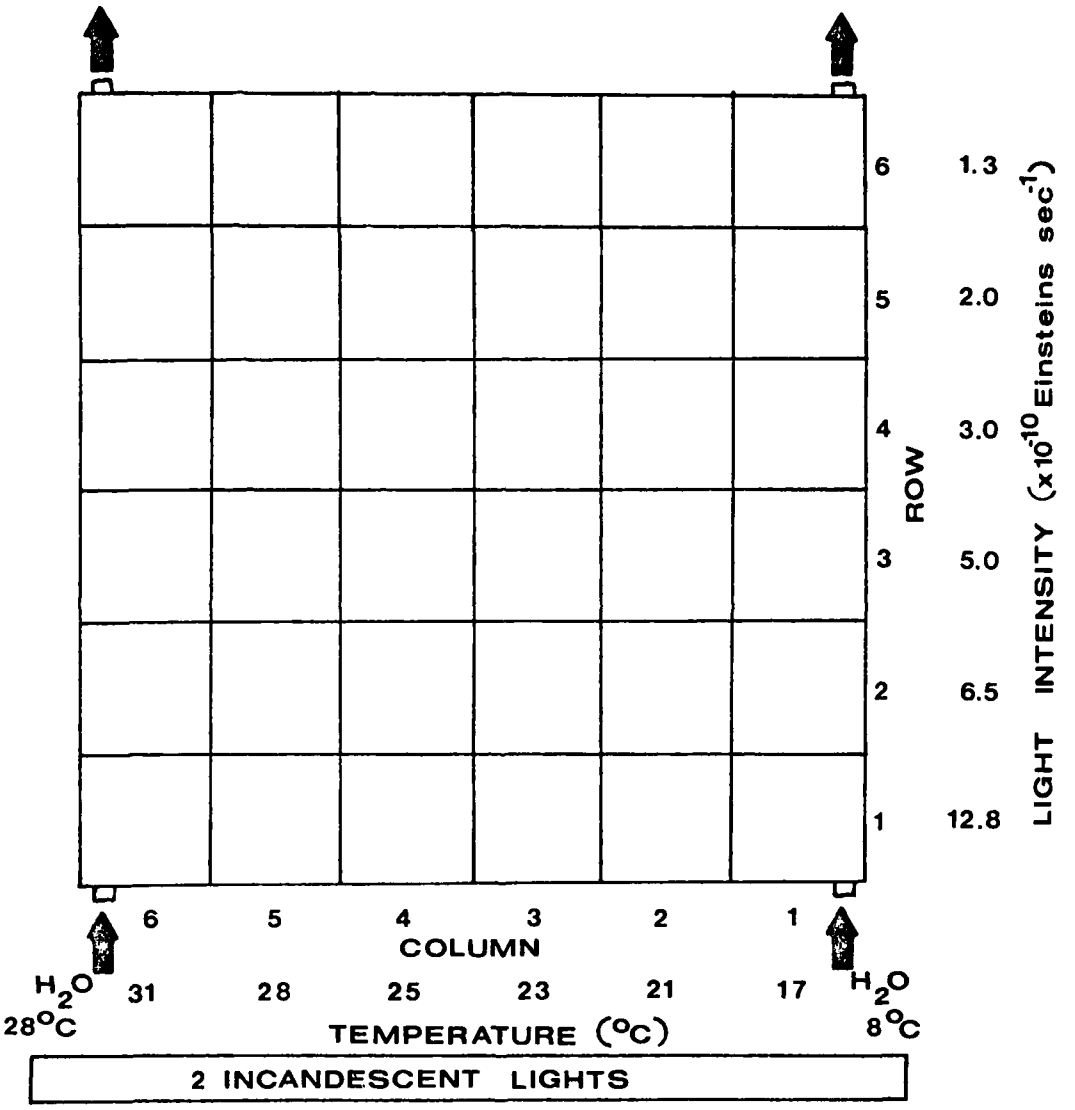

6
5
4
3
2
1
ROW
1.3
2.0
3.0
5.0
6.5
12.8
LIGHT INTENSITY (x10^-10 Einsteins sec^-1)
6
5
4
3
2
1
COLUMN
H2O
28°C
31
28
25
23
21
17
H2O
8°C
TEMPERATURE (°C)
2 INCANDESCENT LIGHTS

Figure 5

Light and Temperature Gradient Plate

The petri plates were arranged on the aluminum plate with temperature indicated by column and light intensity (fluorescent) indicated by row. Temperature was measured in °C. Light intensity was measured in Einsteins sec^{-1} ($x10^{-10}$).

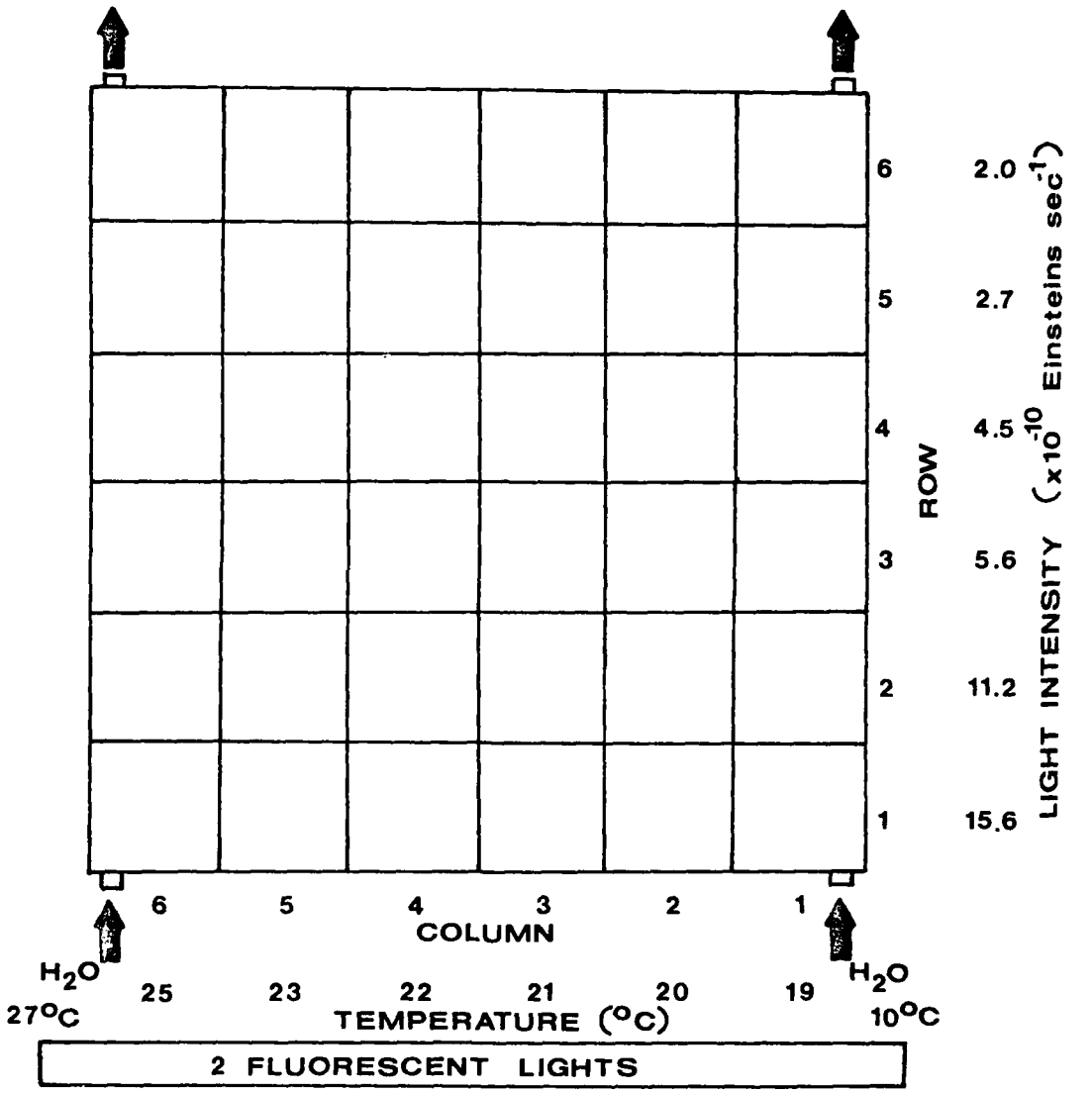

6
5
4
3
2
1
ROW
2.0
2.7
4.5
5.6
11.2
15.6
LIGHT INTENSITY (x10^{-10} Einsteins sec^{-1})
6
5
4
3
2
1
COLUMN
H_2O
27°C
25
23
22
21
20
19
H_2O
10°C
TEMPERATURE (°C)
2 FLUORESCENT LIGHTS

Figure 6A

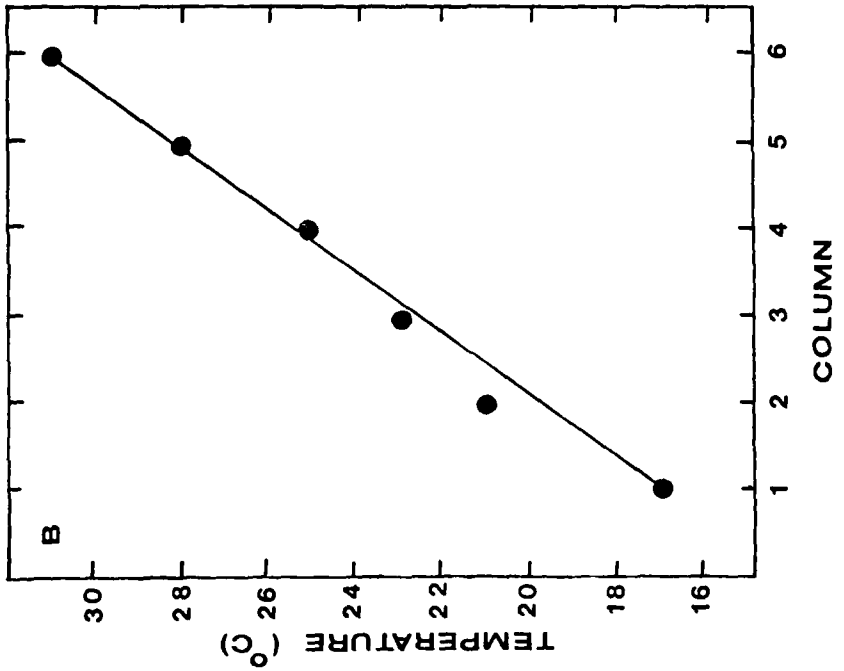
Gradient Curve of Light Intensity

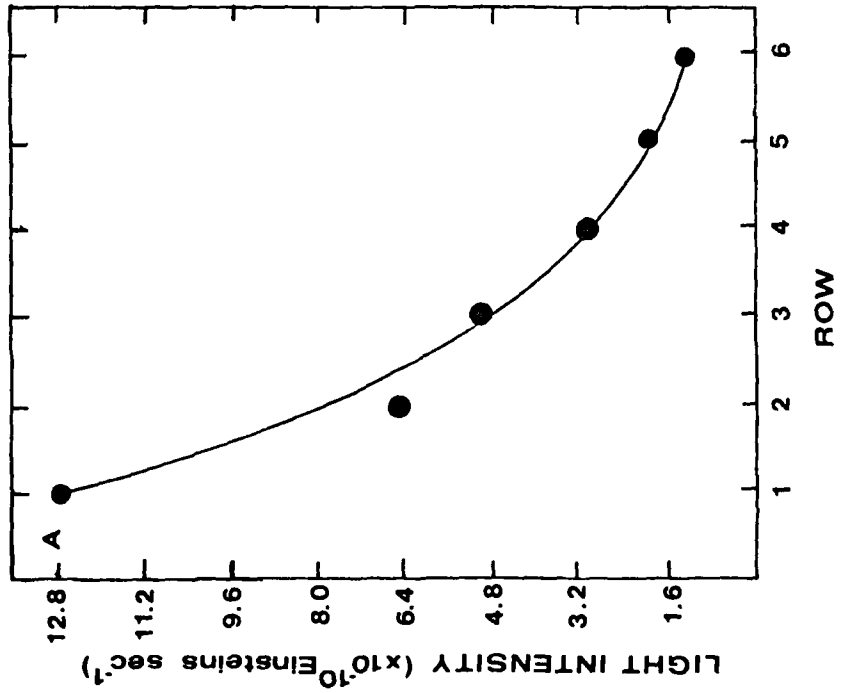
Variation of light intensity (incandescent) with distance on the crossed-gradient plate. The x-axis is distance or row while the y-axis is light intensity, measured in Einsteins $sec^{-1}(x10^{-10})$.

Figure 6B

Gradient Curve of Temperature

Variation of temperature (incandescent) with distance on the crossed-gradient plate. The x-axis is distance or column while the y-axis is temperature, measured in °C.


B
TEMPERATURE (°C)
30
28
26
24
22
20
18
16
COLUMN
1
2
3
4
5
6


A
LIGHT INTENSITY (x10⁻¹⁰Einsteins sec⁻¹)
12.8
11.2
9.6
8.0
6.4
4.8
3.2
1.6
ROW
1
2
3
4
5
6

A range from 17 C to 31°C was obtained. Variation of light intensity with distance for fluorescent lights is shown in Figure 7A. Light intensity decreases in a curvilinear fashion with distance but not as pronounced as under incandescent lights. Also, a higher range of light intensity under the fluorescent lights from 15.6×10^{-10} Einsteins sec^{-1} to 2.0×10^{-10} Einsteins sec^{-1} was obtained. There was no quick drop in light intensity as seen with incandescent lights. Variation of temperature with distance under fluorescent lights is shown in Figure 7B. Temperature increases linearly from Column 1 to Column 6 but not as sharply as under incandescent lights. A range from 19°C to 25°C was obtained. The differences in temperature range and slope of the curves was due to fluorescent lamps being a cooler light than the incandescent lights. Temperature was measured in °C with an Omega Pyrometer, Model 8020. Light intensity was measured in $\mu w\ cm^{-2} m\mu^{-1}$ with an ISCO Spectroradiometer and then converted into Einsteins sec^{-1}. The Spectroradiometer was also used to measure the visible light spectrum for both incandescent and fluorescent lights. The light intensity was measured from 380 nm to 760 nm every 25 nm. The graph of these measurements is shown in Figure 8. The fluorescent lights exhibited a broad shoulder around 425 nm to 525 nm, then peaked at 575 nm where the maximum light intensity was achieved. The light intensity quickly dropped off thereafter. The incandescent lights started at 400 nm and gradually increased in intensity with the maximum at 760 nm.

D. Determination of Medium Components on Adhesion

The effects of various medium components on adhesion were studied by making up medium A and deleting the component that was to be studied.

Figure 7A

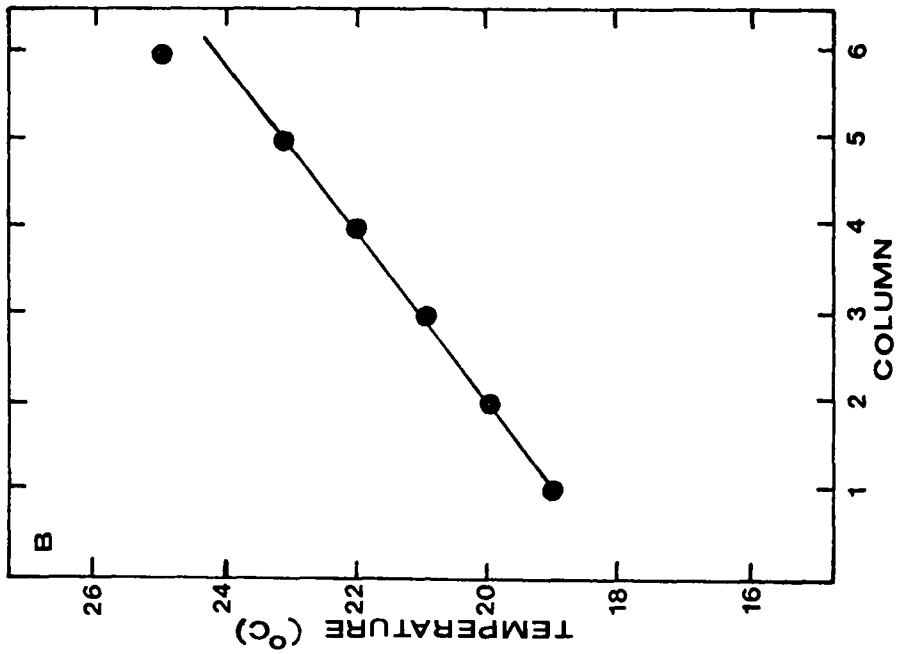
Gradient Curve of Light Intensity

Variation of light intensity (fluorescent) with distance on the crossed-gradient plate. The x-axis is distance or row while the y-axis is light intensity, measured in Einsteins $sec^{-1}(x10^{-10})$.

Figure 7B

Gradient Curve of Temperature

Variation of temperature (fluorescent) with distance on the crossed-gradient plate. The x-axis is distance or column while the y-axis is temperature, measured in °C.


B
TEMPERATURE (°C)
26
24
22
20
18
16
COLUMN
1
2
3
4
5
6

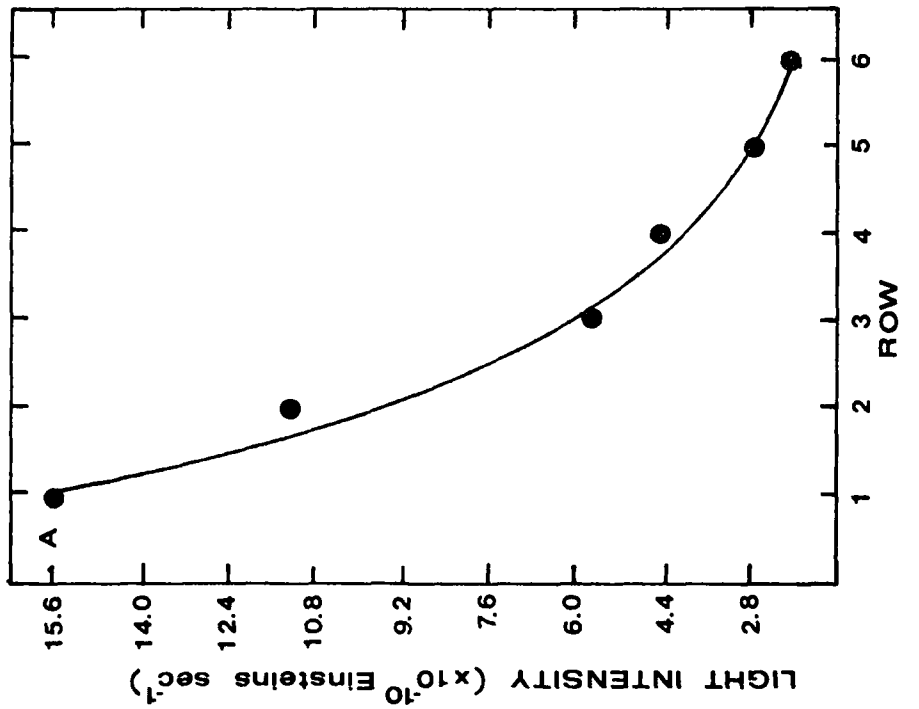
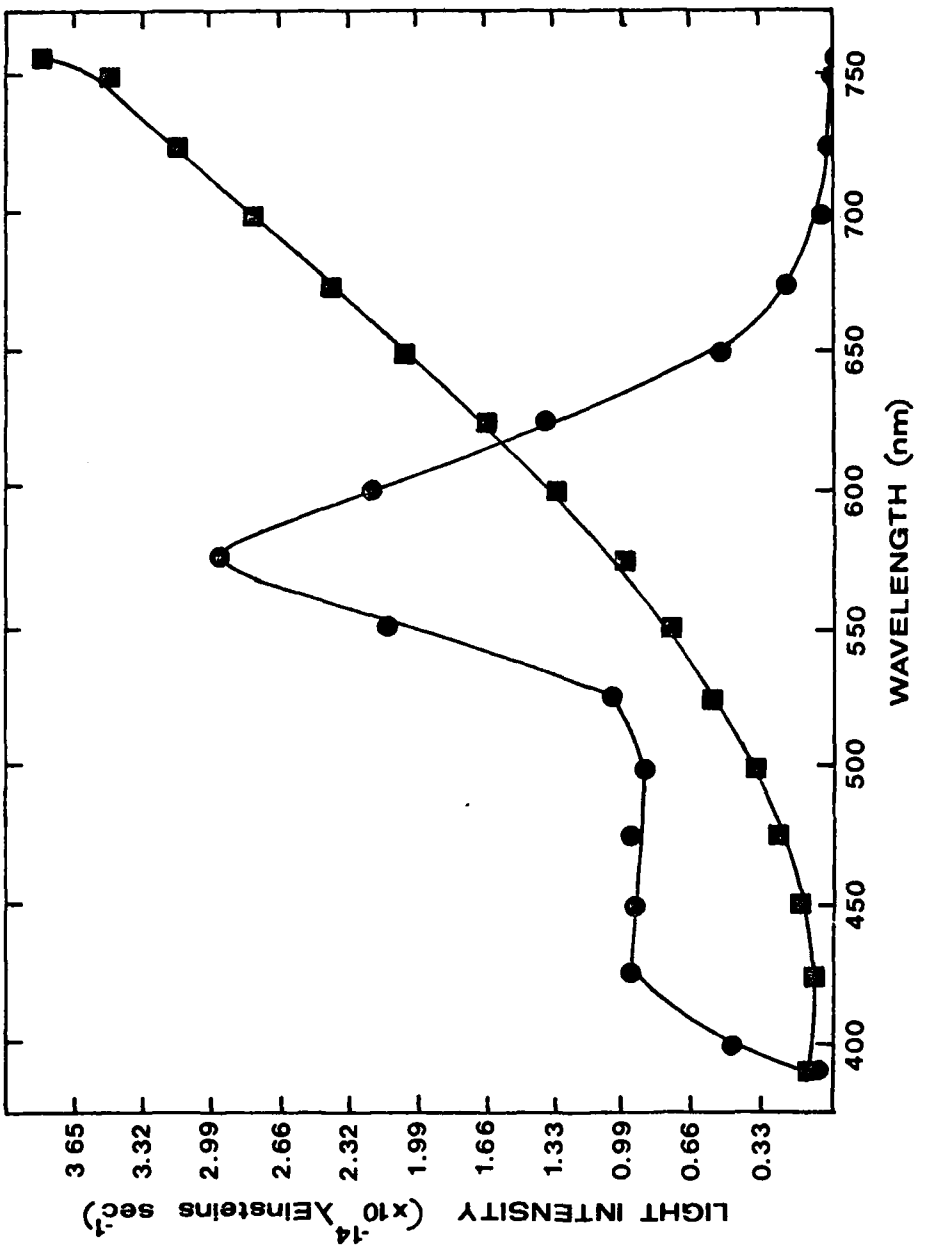

A
LIGHT INTENSITY (x10⁻¹⁰ Einsteins sec⁻¹)
15.6
14.0
12.4
10.8
9.2
7.6
6.0
4.4
2.8
ROW
1
2
3
4
5
6

Figure 8

Visible Light Spectrum

The visible light spectrum produced by fluorescent light (●) and by incandescent light (■) at increasing wavelengths. Light intensity was measured with an ISCO Spectroradiometer at 25 nm intervals. Light intensity was expressed in λEinsteins $sec^{-1}(x10^{-14})$.


LIGHT INTENSITY (x10⁻¹⁴ λEinsteins sec⁻¹)
3.65
3.32
2.99
2.66
2.32
1.99
1.66
1.33
0.99
0.66
0.33
400
450
500
550
600
650
700
750
WAVELENGTH (nm)

This modified medium was added to two-week-old cultures that had been rinsed twice with distilled water. Two days later, all cultures had their percentage of adhesion determined. The effects of calcium on adhesion was investigated in more detail. Two-week-old cultures were washed with distilled water, and calcium-depleted medium A was added. This was repeated every day for three days. On day 4 medium A was renewed each day until day 8. The percentage of adhesion and calcium concentration was measured each day. The effects of 1% Na_2EDTA and HOEDTA on adhesion were also measured. Both these chelators were added to medium A without calcium separately. Two-week-old cultures were washed with distilled water and medium A with one of the chelators added. These effects on adhesion were checked every six hours for 24 hours. Calcium concentration was measured with a Perkin-Elmer 305A Atomic Absorption Spectrophotometer with a Perkin-Elmer Hollow Cathode Intensitron Lamp. The standard was $CaCO_3$. The standard $CaCO_3$ and Na_2EDTA were obtained from Sigma Chemical Company. The chemical HOEDTA (Chel DM Acid) was obtained from Ciba-Geigy Corporation.

E. Determination of DCMU and Enzyme Effects

DCMU (Diuron) 3-(3,4-Dichlorophenyl)-1,1-dimethylurea was used at a concentration of 10^{-5} M. It was prepared in medium A and added to two-week-old cultures which were rinsed twice in distilled water. DCMU was removed on day 4 by repeated washings with distilled water. Medium A was returned to the cultures. Adhesion was measured each day. DCMU was obtained commercially from Agway as Karmex Diuron, then purified.

Trypsin and α-amylase were used in concentrations of 0.1%. They were prepared in medium A which was added to two-week-old cultures that had been rinsed twice in distilled water. Trypsin and α-amylase were obtained from Sigma Chemical Company.

F. Determination of Effects of Various Wavelengths of Light

The effect of various wavelengths of light on adhesion were determined by irradiating the culture for five minutes with a Viewlex slide projector and appropriate filters (Carolina Monochromatic Light Filters, CBS Blue 450 nm, CBS Green 545 nm, CBS Red 650 nm, and CBS Far-Red 750 nm). After irradiation of the culture, it was kept in total darkness for one hour. After one hour dark incubation, the cultures were placed in the environmental incubator and the percentage of adhesion measured on day 3. The filters were obtained from the Carolina Biological Supply Company.

III. RESULTS

A. Effect of Culture Age on Adhesion

The growth of Enteromorpha and the percentage of adhesion plotted against time are shown in Figure 9. Growth was measured as cell dry weight. Percentage of adhesion was determined as described in the section on Materials and Methods. A Biphasic growth response was observed after a lag period of seven days. From seven to 13 days, the generation time was 2.4 days. From 13 to 32 days, the generation time was 6.02 days.

Interestingly, the change in growth rate occurred at a time coincident with establishment of maximal adhesion. Microscopic observations indicated that most of the growth from seven to 12 days was in holdfast development. From 13 to 32 days, most of the growth was associated with thallus development.

The percentage of adhesion increased dramatically from seven to 11 days. At 12 days, 85% adhesion was obtained. During the remainder of the experiment, the percentage of adhesion varied from 85 by ± 5%.

B. The Effect of Temperature and Light on Growth and Adhesion

The growth and percentage of adhesion of Enteromorpha under varying light intensities and temperatures are shown in Table 2 for incandescent light, and in Table 3 for fluorescent light. Light intensity was in Einsteins sec^{-1}. Temperature was in °C. Growth was measured by cell dry weight. Carbon dioxide concentration was that of air and remained constant. A three-dimensional representation of the effect of

Figure 9

Effect of Culture Age on Adhesion

The effect of culture age on adhesion. Growth is log of cell dry weight ($x10^{-3}g$) (●). Adhesion (■) is expressed as % adhesion.

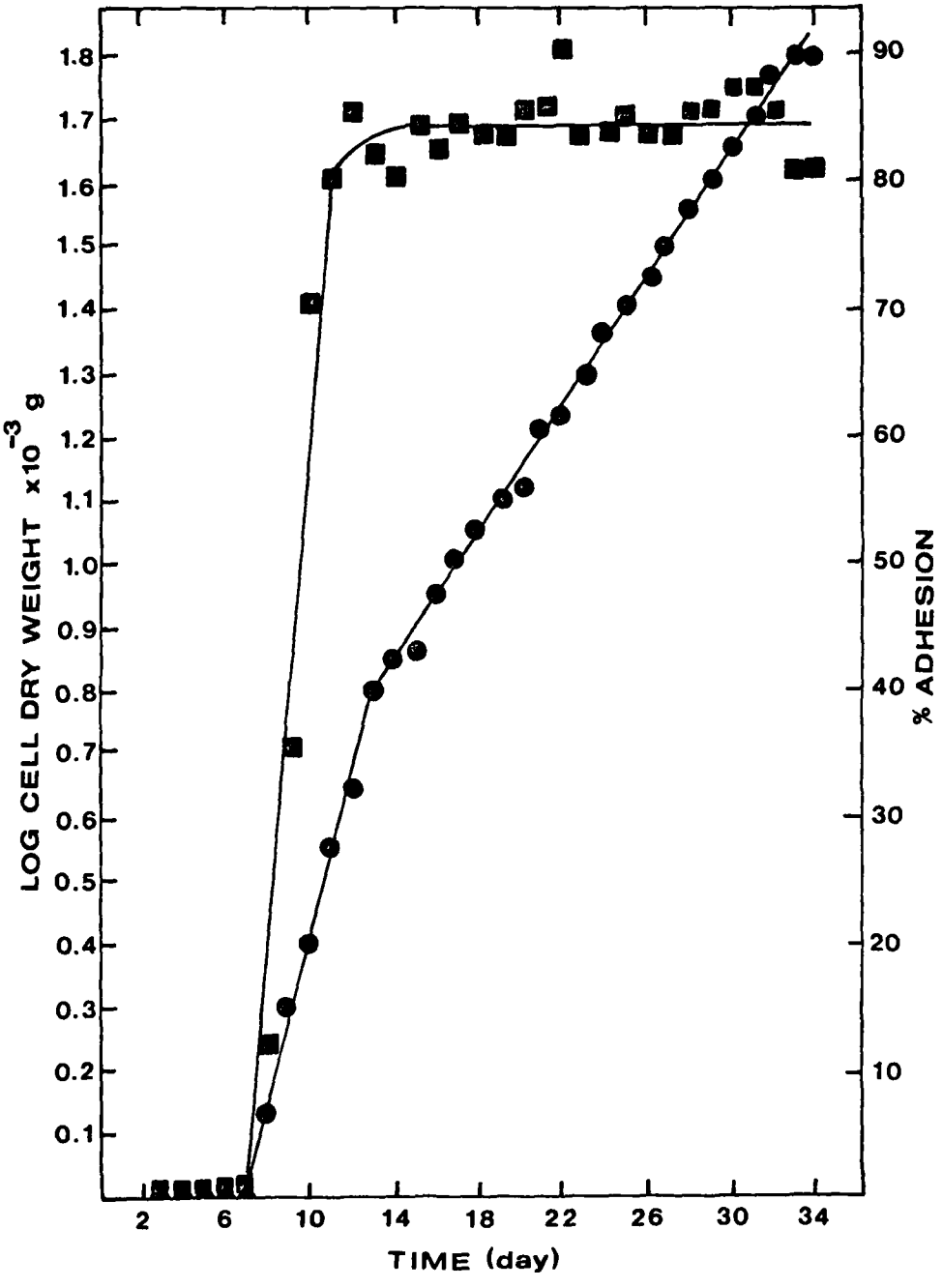

LOG CELL DRY WEIGHT x10^{-3} g
% ADHESION
TIME (day)
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
90
80
70
60
50
40
30
20
10
2
6
10
14
18
22
26
30
34

Table 2

Growth[1] and Adhesion[2] of Enteromorpha under Varying Light Intensity[3] and Temperature[4] for Incandescent Lights

Row	Column	1	2	3	4	5	6
1	Light Intensity	12.8	12.8	12.8	12.8	12.8	12.8
	Temperature	17	21	23	25	28	31
	Growth	7	23	31	31	30	26
	Adhesion	1	13	19	10	1	1
2	Light Intensity	6.5	6.5	6.5	6.5	6.5	6.5
	Temperature	17	21	23	25	28	31
	Growth	17	26	28	31	29	22
	Adhesion	1	16	28	14	2	1
3	Light Intensity	5.0	5.0	5.0	5.0	5.0	5.0
	Temperature	17	21	23	25	28	31
	Growth	7	13	25	29	29	20
	Adhesion	2	51	89	19	4	2
4	Light Intensity	3.0	3.0	3.0	3.0	3.0	3.0
	Temperature	17	21	23	25	28	31
	Growth	5	11	24	25	23	16
	Adhesion	1	32	46	3	2	1
5	Light Intensity	2.0	2.0	2.0	2.0	2.0	2.0
	Temperature	17	21	23	25	28	31
	Growth	2	6	6	7	14	14
	Adhesion	1	8	35	3	1	1
6	Light Intensity	1.3	1.3	1.3	1.3	1.3	1.3
	Temperature	17	21	23	25	28	31
	Growth	1	2	3	4	14	12
	Adhesion	1	2	11	2	2	1

[1]Measured by cell dry weight ($x10^{-3}g$).

[2]Measured in % adhesion.

[3]Measured in Einsteins sec^{-1} ($x10^{-10}$)

[4]Measured in °C.

Table 3

Growth[1] and Adhesion[2] of Enteromorpha under Varying Light Intensity[3] and Temperature[4] for Fluorescent Lights

Row	Column	1	2	3	4	5	6
1	Light Intensity	15.6	15.6	15.6	15.6	15.6	15.6
	Temperature	19	20	21	22	23	25
	Growth	6	10	28	21	7	4
	Adhesion	54	75	72	66	44	11
2	Light Intensity	11.2	11.2	11.2	11.2	11.2	11.2
	Temperature	19	20	21	22	23	25
	Growth	5	11	29	23	7	3
	Adhesion	50	72	66	57	21	7
3	Light Intensity	5.6	5.6	5.6	5.6	5.6	5.6
	Temperature	19	20	21	22	23	25
	Growth	4	10	17	13	7	3
	Adhesion	15	54	53	50	17	3
4	Light Intensity	4.5	4.5	4.5	4.5	4.5	4.5
	Temperature	19	20	21	22	23	25
	Growth	4	11	14	13	5	2
	Adhesion	11	44	45	45	8	1
5	Light Intensity	2.7	2.7	2.7	2.7	2.7	2.7
	Temperature	19	20	21	22	23	25
	Growth	2	6	10	9	2	1
	Adhesion	8	39	40	39	8	0
6	Light Intensity	2.0	2.0	2.0	2.0	2.0	2.0
	Temperature	19	20	21	22	[illegible]	25
	Growth	2	2	8	4	1	0
	Adhesion	0	0	1	0	0	0

[1]Measured by cell dry weight ($x10^{-3}$g).

[2]Measured in % adhesion.

[3]Measured in Einsteins sec^{-1} ($x10^{-10}$)

[4]Measured in °C.

temperature and light intensity for incandescent light is shown in Figure 10 and for fluorescent light in Figure 11.

Under both types of light, growth increased with an increase in light intensity. At low temperature and low light intensity, growth was slowest under both incandescent and fluorescent light. However, under fluorescent light, growth was also slow at the higher temperatures (Figure 11). Under incandescent light, growth leveled off and remained stationary at 6.43×10^{-10} Einsteins sec^{-1} (Figure 10). Under fluorescent light, growth did not level off until a light intensity of 1.11×10^{-9} Einsteins sec^{-1} was reached (Figure 11). A dramatic increase in growth was seen after a light intensity of 5.56×10^{-10} Einsteins sec^{-1} was reached with fluorescent light (Figure 11), while under incandescent light (Figure 10), this increase in growth was seen after 1.98×10^{-10} Einsteins sec^{-1} was reached.

As the temperature rose, the growth increased until an optimum temperature of 25°C at high light intensity and 28°C at low light intensity was reached using incandescent light (Figure 10). Except at the highest light intensity, growth began to decrease slightly with increasing temperatures past the optimum. Under fluorescent light (Figure 11), the temperature effect was quite different. There was a sharp peak of optimum growth at 21°C with a rapid decline on either side of this temperature at all light intensities.

The effect of temperature and light intensity on adhesion is presented as a three-dimensional representation for incandescent light in Figure 12 and for fluorescent light in Figure 13. The effects of the two kinds of light were more varied on the adhesion of Enteromorpha than that observed for growth. Maximum adhesion of 89% was obtained

Figure 10

A Three-dimensional Display of the Effects of Temperature and Light Intensity (Incandescent Light) On Growth

Growth is plotted on the vertical axis and was measured as cell dry weight ($x10^{-3}g$). Temperature (°C) and light intensity (Einsteins sec^{-1} x 10^{-10}) are plotted perpindicular to growth.

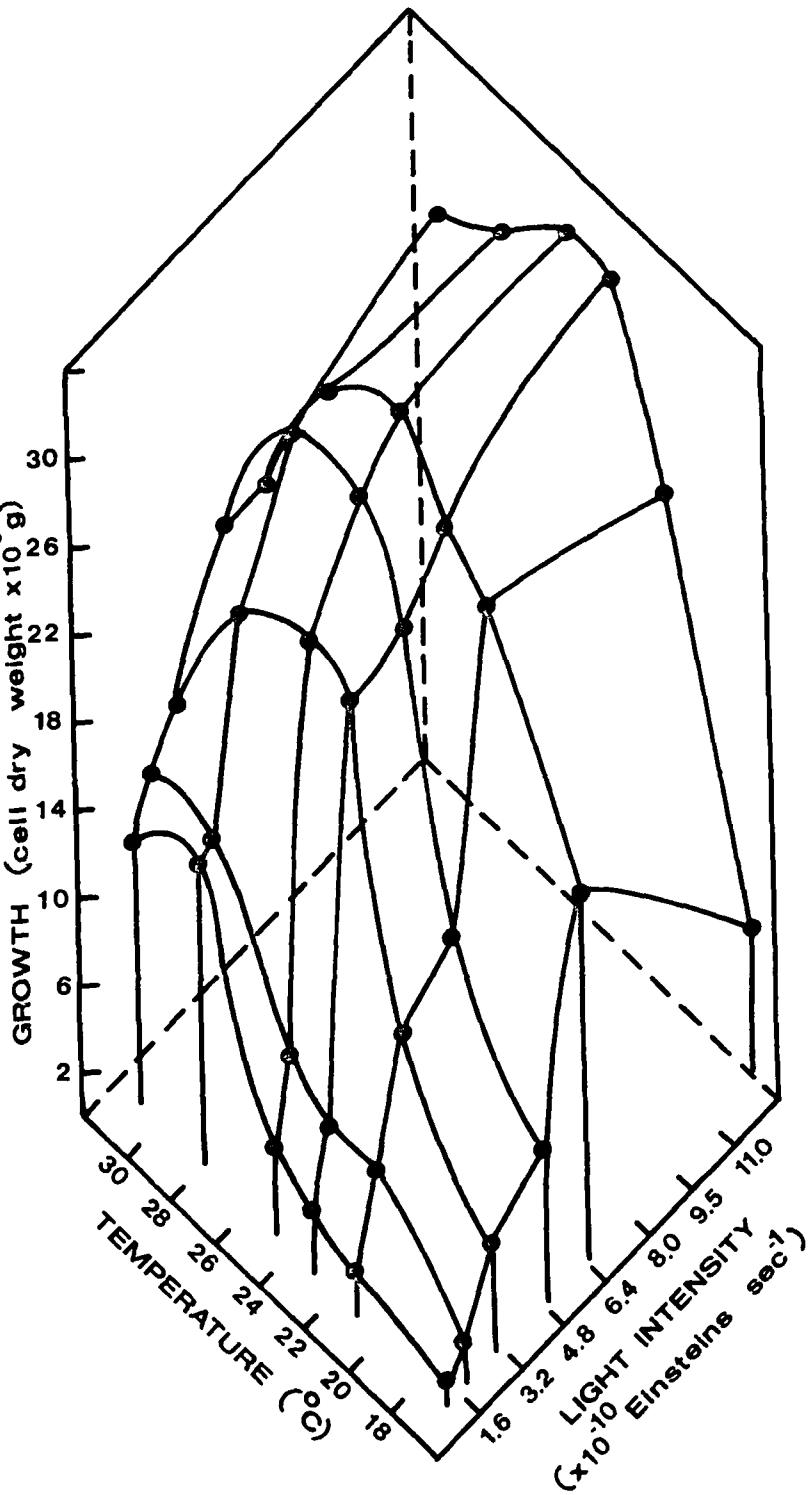

GROWTH (cell dry weight x10^-3 g)
30
26
22
18
14
10
6
2
TEMPERATURE (°C)
30 28 26 24 22 20 18
LIGHT INTENSITY
(x10^10 Einsteins sec^-1)
1.6 3.2 4.8 6.4 8.0 9.5 11.0

Figure 11

A Three-dimensional Display of the Effects of Temperature and Light Intensity (Fluorescent Light) On Growth

Growth is plotted on the vertical axis and was measured as cell dry weight ($x10^{-3}g$). Temperature (°C) and light intensity (Einsteins sec^{-1} x 10^{-10}) are plotted perpindicular to growth.

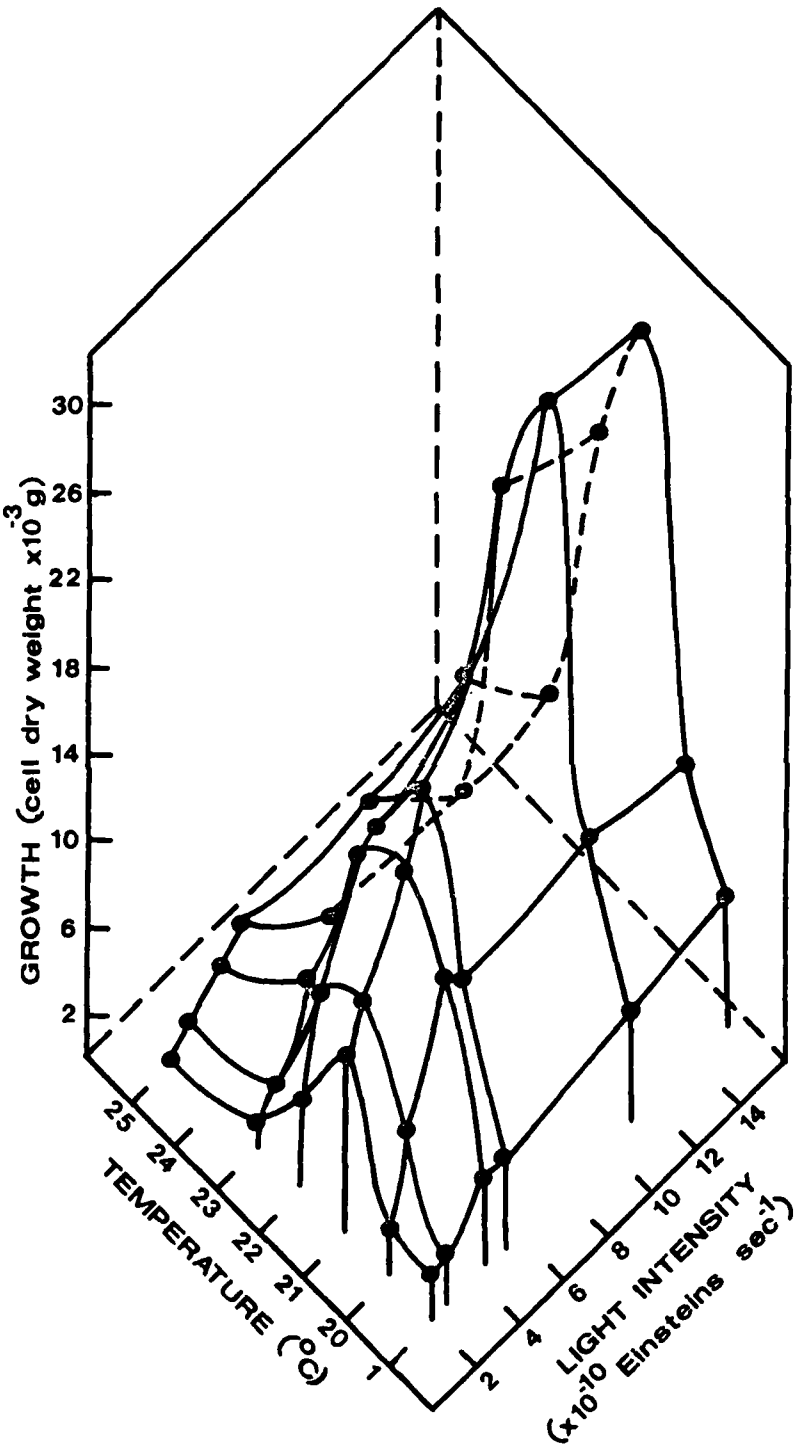

GROWTH (cell dry weight x10^-3 g)
30
26
22
18
14
10
6
2
TEMPERATURE (°C)
25
24
23
22
21
20
1
LIGHT INTENSITY
(x10^-10 Einsteins sec^-1)
2
4
6
8
10
12
14

Figure 12

A Three-dimensional Display of the Effects of Temperature and Light Intensity (Incandescent Light) On Adhesion

Adhesion is plotted on the vertical axis and was measured as % adhesion. Temperature (°C) and light intensity (Einsteins $sec^{-1} \times 10^{-10}$) are plotted perpindicular to adhesion.

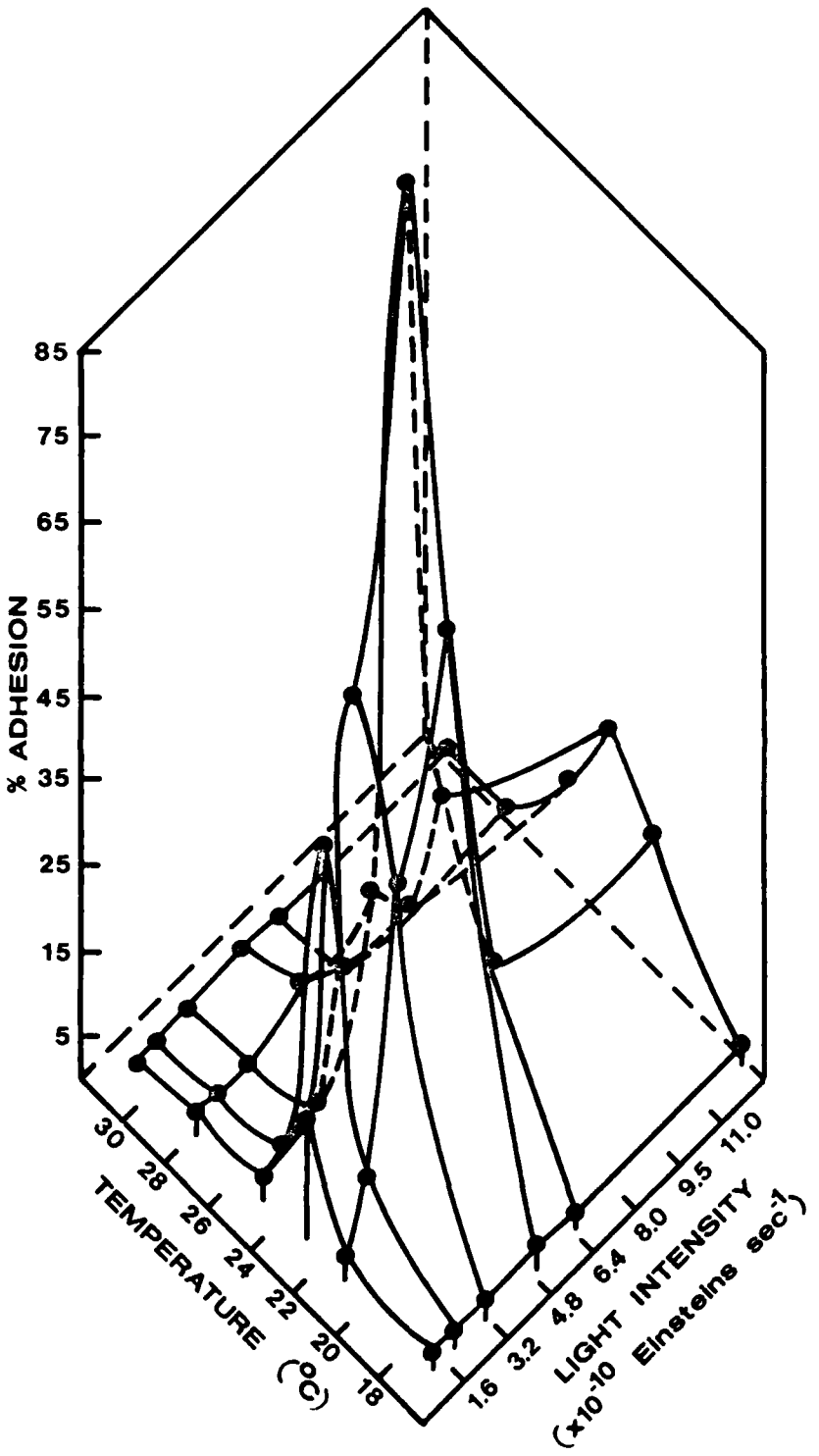
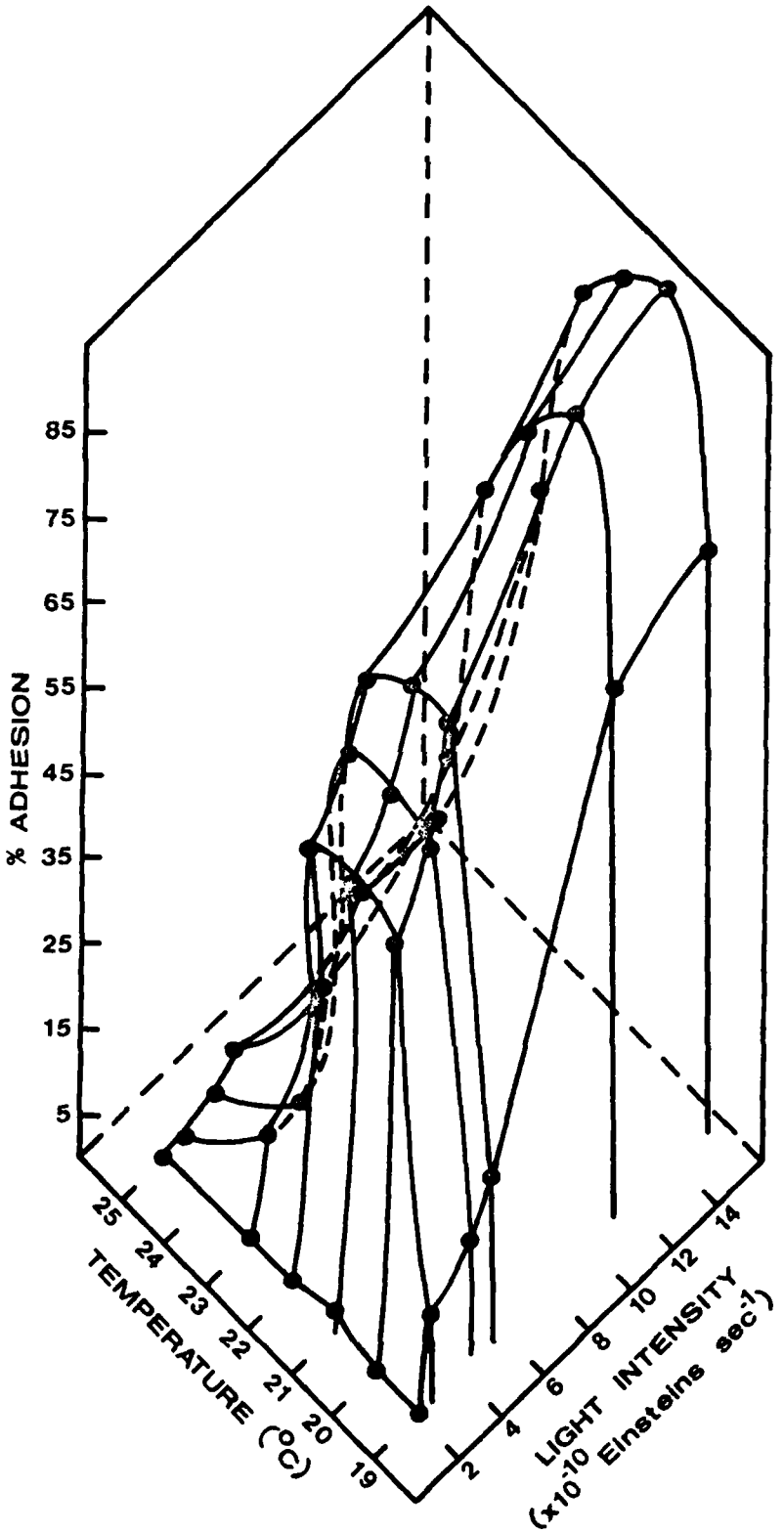

% ADHESION
85
75
65
55
45
35
25
15
5
TEMPERATURE (°C)
30 28 26 24 22 20 18
LIGHT INTENSITY
(x10⁻¹⁰ Einsteins sec⁻¹)
1.6 3.2 4.8 6.4 8.0 9.5 11.0

Figure 13

A Three-dimensional Display of the Effects of Temperature and Light Intensity (Fluorescent Light) On Adhesion

Adhesion is plotted on the vertical axis and was measured as % adhesion. Temperature (°C) and light intensity (Einsteins $sec^{-1} \times 10^{-10}$) are plotted perpindicular to adhesion.


% ADHESION
85
75
65
55
45
35
25
15
5
TEMPERATURE (°C)
25
24
23
22
21
20
19
LIGHT INTENSITY
(x10⁻¹⁰ Einsteins sec⁻¹)
2
4
6
8
10
12
14

under incandescent light (Figure 12) at a mid-light intensity range of 6.43×10^{-10} Einsteins sec^{-1} with a sharp decrease before and after this range. Under fluorescent light (Figure 13), adhesion continued to increase up to 72% with an increase in light intensity.

Adhesion increased with an increase in temperature up to an optimum temperature, then decreased under both types of light. However, under incandescent light (Figure 12), the optimum temperature was 23°C with a very sharp drop on either side. Under fluorescent light (Figure 13), adhesion exhibited a broader temperature optimum range of 20°-23°C with a sharp decrease on either side.

C. Effect of Photosynthesis on Adhesion

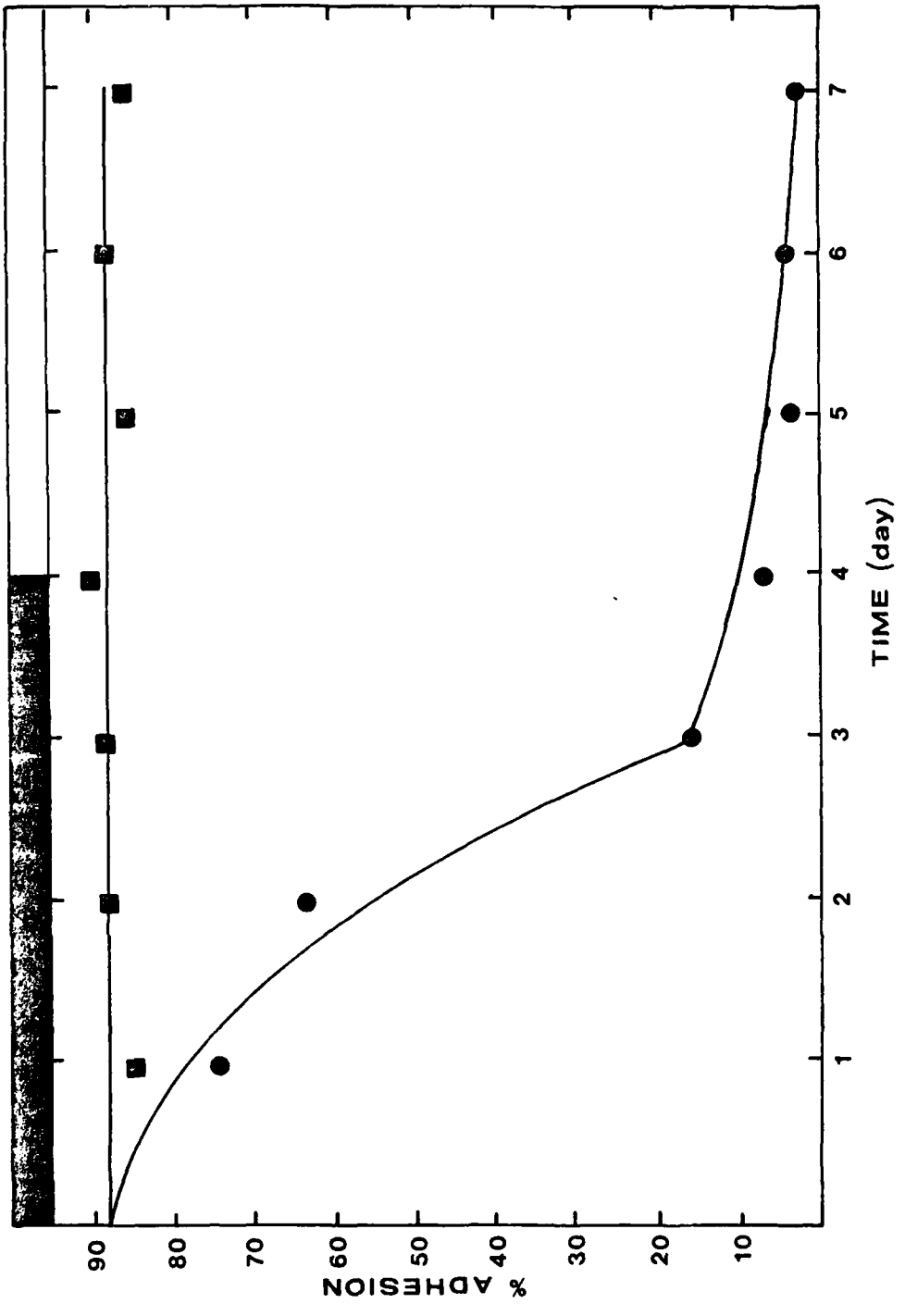
Since light intensity affected adhesion, there might be a correlation between adhesion and rate of photosynthesis. The rate of photosynthesis can be markedly affected by light intensity. To look at the effect photosynthesis might have on adhesion, I decided to inhibit photosynthesis. One way to inhibit photosynthesis is to remove the source that drives the photochemical reaction, namely, light. Two-week-old cultures were subjected to various periods of darkness and then the percentage of adhesion was measured. As shown in Figure 14, Enteromorpha's adhesion to glass decreased with time. By day 4, there was essentially no adhesion left. If day 4 dark incubated cultures were then exposed to light, adhesion did not return with time.

To try to determine whether the loss in attachment was due to some kind of photochemical reaction that affected the adhesive material or was a result of the loss in production of energy-yielding compounds due to the shutdown of the photosynthetic apparatus, DCMU (10^{-5} M) was

Figure 14

Effect of Darkness on Adhesion with Time

At time 0, two-week-old cultures were wrapped in foil. On the fourth day, all remaining cultures were restored to light. The control cultures remained in light (■), while all other cultures were subject to darkness (●).


% ADHESION
90
80
70
60
50
40
30
20
10
TIME (day)
1
2
3
4
5
6
7

added to the culture medium of two-week-old cultures. DCMU is an inhibitor of oxygen evolving photosynthesis through a specific inhibition of photosystem II. As shown in Figure 15, the effect of DCMU was the same as the effect of darkness on adhesion. There was a decrease in adhesion with time of exposure to DCMU. By day 4, there was essentially no adhesion left. As in the effect of no light, adhesion did not return to four-day-old treated cultures when DCMU was removed.

D. Effect of Different Wavelengths of Light on Adhesion

Light of specific wavelengths has been shown to induce adhesion by Tanada in root tips (21,22) and Nagata in Spirogyra (12). To find out if adhesion was affected by a specific wavelength of light, two-week-old cultures were exposed to specific wavelengths of light. The effects of four wavelengths of light are shown in Figure 16. Of the four wavelengths used, far-red (750 nm) produced the most dramatic effect on adhesion. Red light (650 nm) had no effect. Blue light (450 nm) and green light (545 nm) produced only a slight effect. Since the red and far-red light affected adhesion oppositely, this suggests a photochrome effect. One of the methods for showing that a phytochrome system is operating is by showing a red/far-red reversible nature of some physiological response. The response in this case was adhesion. The effect of alternating exposure to red and far-red is shown in Table 4. In each case, adhesion was lost when the last exposure was by far-red, while adhesion was not affected when the last exposure was by red light. This suggested that the loss of adhesion was somehow related to a phytochrome system.

Figure 15

Effect of DCMU (10^{-5} M) on Adhesion with Time

At time 0, two-week-old cultures had DCMU added. On the fourth day, all remaining cultures were repeatedly washed to remove DCMU and medium A was added. Control cultures (■) and cultures subject to DCMU (●).

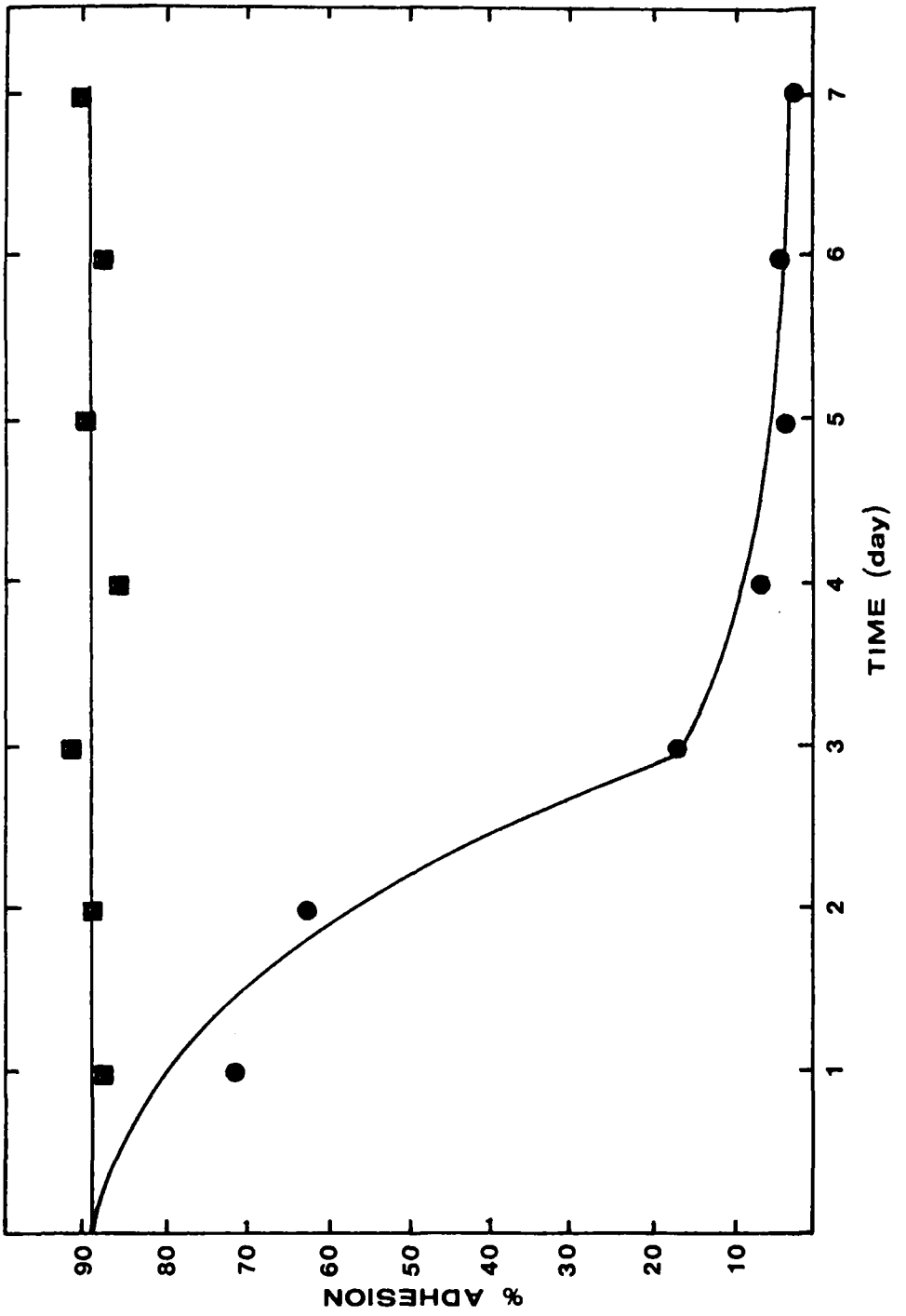

% ADHESION
90
80
70
60
50
40
30
20
10
TIME (day)
1
2
3
4
5
6
7

Figure 16

Effect of Five-minute Irradiation of
Different Wavelengths of Light on Adhesion

After irradiation, all cultures including the control were incubated in the dark for one hour. After the one-hour incubation, the cultures were inclubated under fluorescent light and the percentage of adhesion was measured on day 3.

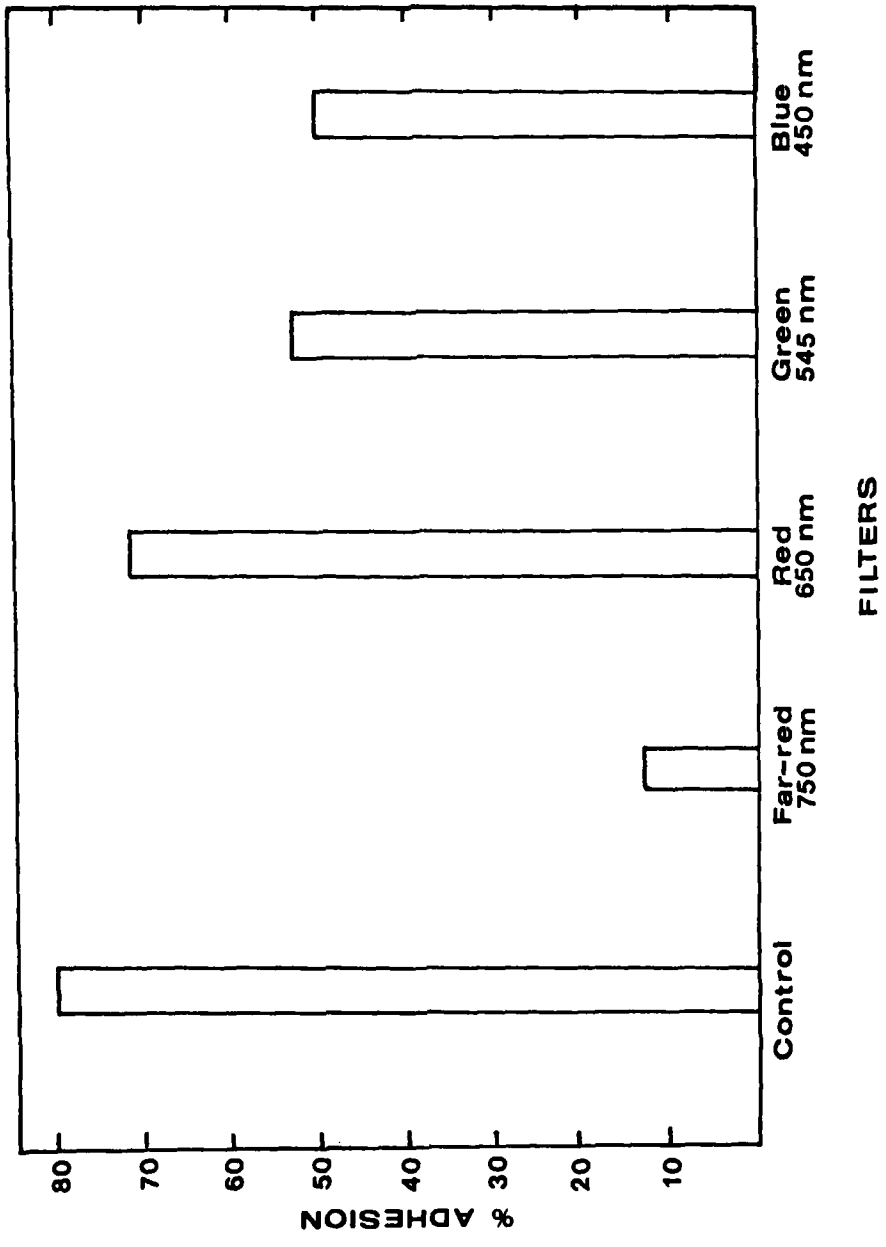

% ADHESION
80
70
60
50
40
30
20
10
Control
Far-red 750 nm
Red 650 nm
Green 545 nm
Blue 450 nm
FILTERS

Table 4

Effect of Red and Far-Red Light on Adhesion[1]

Treatment	% Adhesion
Control	79
Red (1 min)	79
Far-Red (1 min)	12
Red (1 min)/Far-Red (1 min)	8
Far-Red (1 min)/Red (1 min)	78
Red (1 min)/Far-Red (1 min)/Red (1 min)	82
Far-Red (1 min)/Red (1 min)/Far-Red (1 min)	13

[1]Two-week-old cultures were irradiated with red light and/or with far-red light for 1 min. Alternate irradiation with red and far-red light was carried out in immediate succession. After irradiation, the cultures were incubated for 1 hour in the dark then incubated for 3 days under fluorescent light. On day 3, the percentage of adhesion was measured.

E. Effect of Medium Components on Adhesion

To find out if medium components were an integral part of the adhesive matrix in Enteromorpha, experiments were run by deleting various components from the medium of two-week-old cultures (Table 5). Of all the components in medium A that were tested, only calcium showed any appreciable effect (Figure 17). The longer the cultures were kept in the medium without calcium, the greater the decrease in adhesion. Unlike the loss of adhesion due to darkness or DCMU, the loss of adhesion due to calcium depletion was restored by adding calcium back into the medium. Two-week-old cultures had medium A removed and replaced with calcium-depleted medium A on day 14. The concentration of calcium in medium A of 14-day-old cultures was 85 mg/l. Each day, the calcium-depleted medium A was replaced with fresh calcium depleted-medium A. On day 18, there was only 2% adhesion and calcium concentration in the medium was zero. On day 18, these calcium-depleted cultures had calcium restored which caused adhesion to be restored.

If a metal chelator such as Na_2EDTA or HOEDTA was added to calcium-depleted medium A, the adhesion was lost at a greater rate as shown in Figure 18. These chelators removed the calcium from the adhesive matrix faster than simple diffusion. HOEDTA is a metal chelator that is preferential for calcium. There was no difference between the effect of HOEDTA and Na_2EDTA on adhesion.

F. Effect of α-amylase and Trypsin on Adhesion

To determine whether the adhesive material of the holdfast structure of Enteromorpha was affected the same way as newly-settled zoospores or already established zoospores (5) α-amylase and trypsin were added to

Table 5

Effect of Deletion of Medium Components on Adhesion[1]

Components Deleted	% Adhesion
$CaCl_2 2H_2O$	10
Na_2EDTA	60
P-1 Metals	58
KCl	64
$FeCl_3 6H_2O$	70
$MgSO_4 7H_2O$	60
NaCl	66
$NaNO_3$	50

[1]Medium A was removed from two-week-old cultures and the cultures were rinsed in distilled water. The modified medium A with the deleted component was added and, on day 2, the percentage of adhesion was measured.

Figure 17

Effect of Calcium Depletion on Adhesion with Time

On day 14, cultures had the old medium removed and calcium-depleted medium A added. This was repeated for days 15, 16, and 17. On day 18, medium A (with calcium) was added and this was repeated for days 19, 20, 21, and 22. Adhesion and calcium concentration were measured concurrently. The control cultures' medium was always replaced with medium A. Each control culture was measured for adhesion (O) and calcium concentration (□). Each culture which was subject to calcium depletion was measured for adhesion (●) and calcium concentration (■).

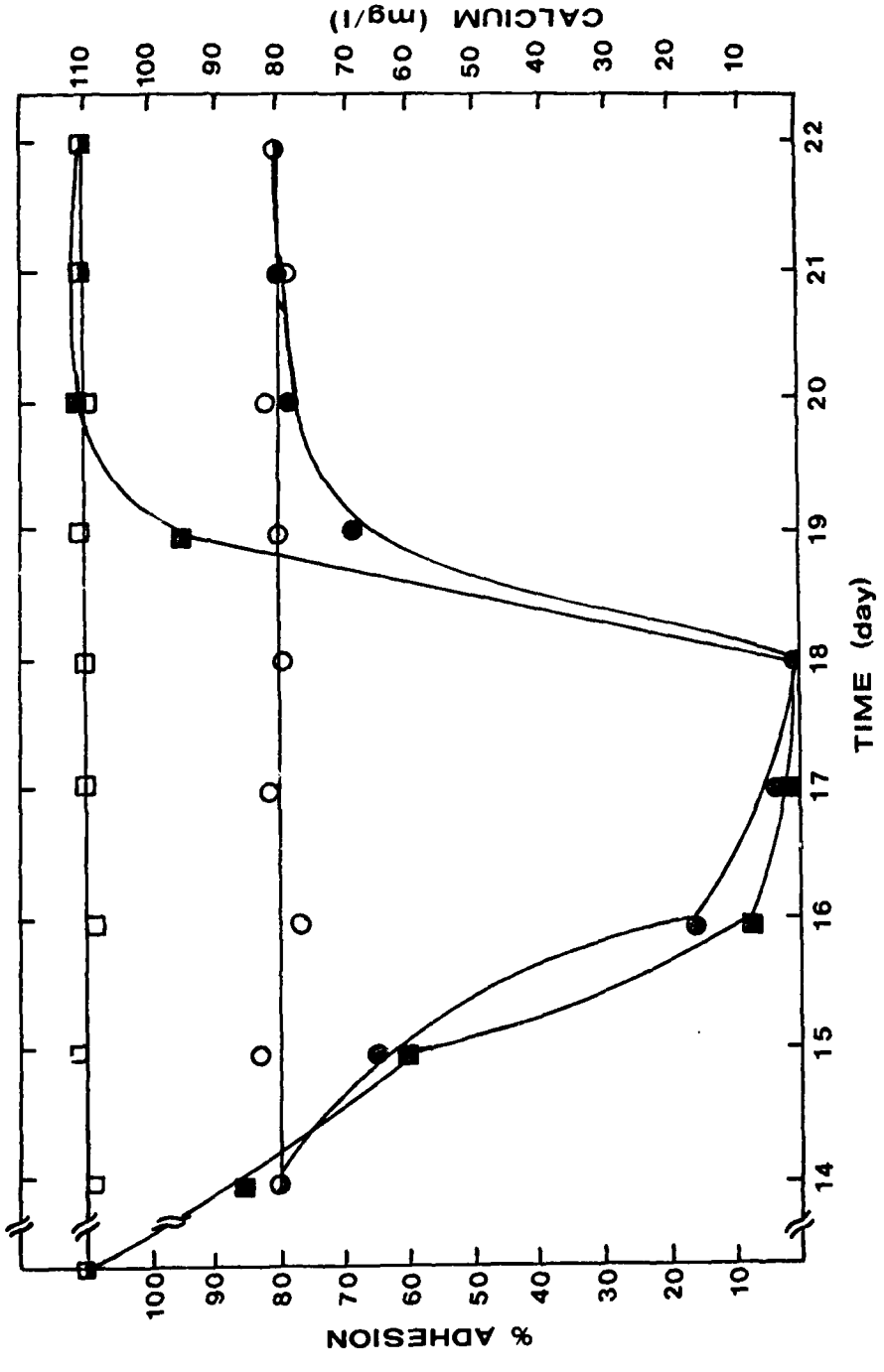
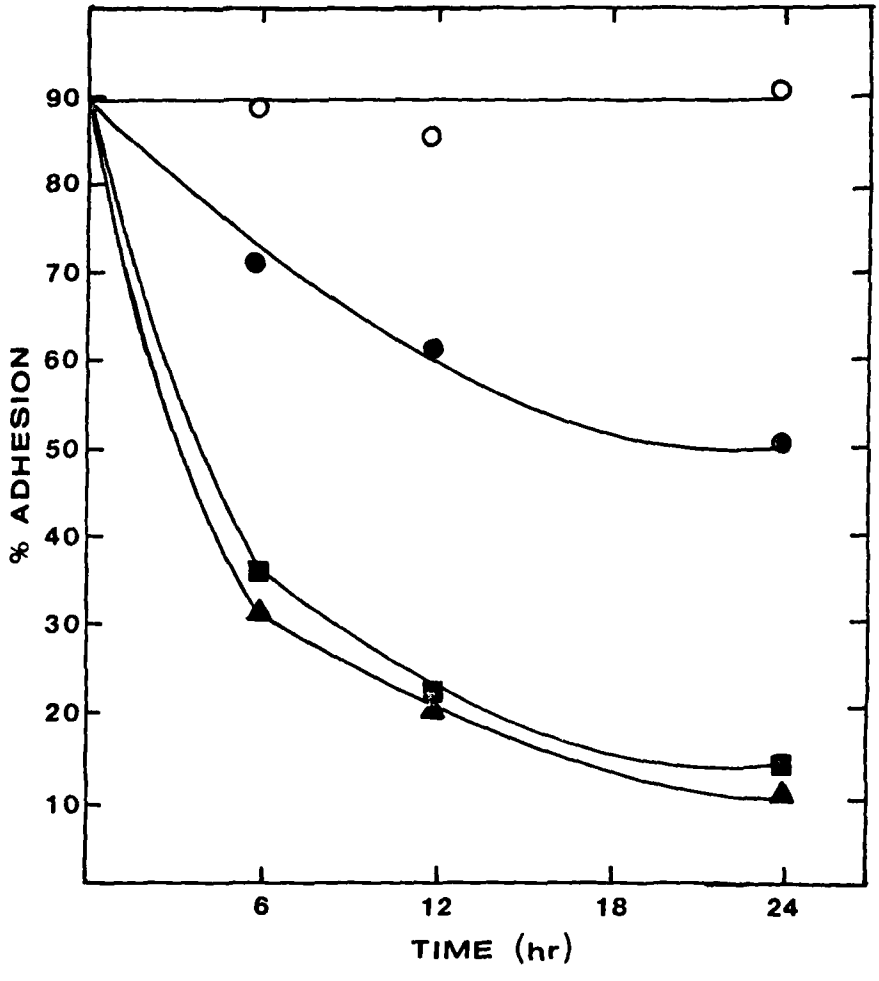

CALCIUM (mg/l)
110
100
90
80
70
60
50
40
30
20
10
22
21
20
19
18
17
16
15
14
TIME (day)
% ADHESION
100
90
80
70
60
50
40
30
20
10

Figure 18

Effect of Calcium Depletion,
Na_2EDTA, and HOEDTA on Adhesion with Time

Control cultures (○), calcium removed at time zero (●), Na_2EDTA added to cultures at time zero (■), and HOEDTA added to cultures at time zero (▲).

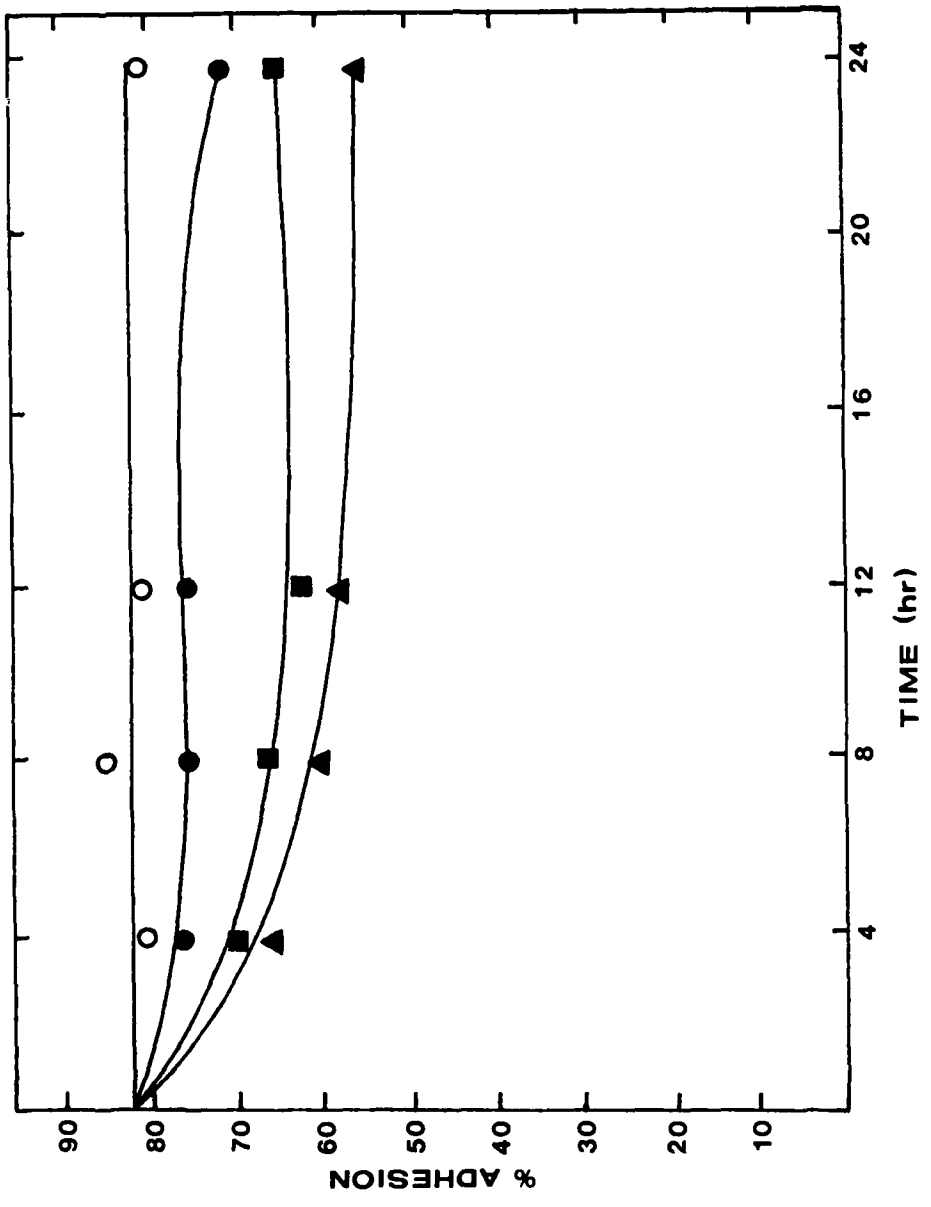

90
80
70
60
50
40
30
20
10
% ADHESION
6
12
18
24
TIME (hr)

two-week-old cultures. The results of these two enzymes on the adhesiveness of the holdfast are shown in Figure 19. As in other reports (5), the activity of these enzymes, whether separately or together, had only a slight effect on adhesion of already established holdfast structures.

Figure 19

Effect of α-amylase and Trypsin Separately or Combined
(both at 0.1 g/100 ml) on the Adhesion of Two-Week-Old Cultures

The enzymes were added to medium A which replaced the old medium.
Control (○); α-amylase (●); trypsin (■); and α-amylase/trypsin (▲).


% ADHESION
90
80
70
60
50
40
30
20
10
TIME (hr)
4
8
12
16
20
24

IV. DISCUSSION

The growth curve in Figure 9 and microscopic observation revealed several interesting things about the growth and adhesion of Enteromorpha. During the lag period from one to seven days, the cells from the homogenized algae stock were preparing to undergo regeneration. From seven to 13 days, there was rapid cell growth as indicated by the steep slope and a generation time of 2.4 days. During this period, the cells were developing into holdfast structures. Along with the holdfast development, the percentage of adhesion was increasing. This would be due to a larger surface area of attachment by the growing holdfast. By day 12, a maximum adhesion of 85% had been reached. There was no thallus development during this period.

After the short period of fast growth, the developing algal plant's growth slowed down. This was indicated by a change in slope from day 13 to day 32. The generation time during this period was 6.02 days. It was during this time that the thallus structure started to develop. The development of the holdfast was slowing down and eventually reached a point where there was no longer any outward growth. The stopping of outward growth appeared to be a response to crowding. The holdfast of one alga did not crowd onto the holdfast of another alga. This non-crowding effect could be a result of an inhibitory substance in the adhesive matrix. The adhesion remained the same within ± 5%. Thus, adhesion was not lost as the culture got older.

The reason for byphasic growth is unknown. The initial rapid growth might be a survival mechanism in that the cells must attach and develop a holdfast in a relatively short period of time, so it can support the

developing thallus. The biphasic growth was not due to a lack of certain nutrients, for the generation time did not increase when fresh medium was added. However, the alga itself might be producing a growth inhibitor.

Of all the processes in nature, photosynthesis is perhaps the most fundamental. While transferring the free energy of sunlight, the green algae combine carbon dioxide and water and from them builds carbohydrates, which directly or indirectly serves as the source of free energy and growth. Thus, those factors that affect the rate of photosynthesis will affect the rate of growth. The primary reaction in photosynthesis is a photochemical reaction which is dependent on light intensity only. The rate at which chlorophyll molecules absorb light quanta is not increased by raising the temperature. Further, the efficiency of energy transfer from chlorophyll b to chlorophyll a in algae is not affected by temperature. However, there was found to be a slight temperature effect in the energy transfer from the various forms of chlorophyll a to the reaction center (20). The reactions that follow the photochemical reactions are rate dependent on temperature.

Two types of light were used to measure the effects of temperature and light intensity on growth and adhesion. The spectral output of these lights is shown in Figure 8. The wavelengths of visible light that are necessary for photosynthesis are in the red region between 640 nm and 690 nm, and the blue region between 410 nm and 500 nm. If one compares the spectral output of the two types of light in these two regions, the incandescent lights will have a slightly larger incident light intensity. Incandescent light provides an incident light intensity in these regions of 8.61×10^{-10} Einsteins sec^{-1}, while the fluorescent light provides

5.2×10^{-10} Einsteins sec^{-1}. Thus, the incandescent lights supply slightly more light for photosynthesis than does the fluorescent light. One would expect that, under incandescent light, there would be more growth at lower light intensities than under fluorescent light at the same intensity. This was clearly shown to be the case in Figures 10 and 11. Under incandescent light, maximum growth was achieved at 6.43×10^{-10} Einsteins sec^{-1}, while under fluorescent, it was not reached until 1.11×10^{-9} Einsteins sec^{-1}.

The effects of temperature on growth under the two kinds of lights are unexplainable at present. As stated previously, the reactions that follow the photochemical reactions are rate-dependent on temperature. However, this does not explain why, under incandescent light, there is a broad temperature range for maximum growth, but under fluorescent light a very narrow range was observed. Furthermore, the temperature range was different. Under incandescent light, it was between 23°C and 28°C, while under fluorescent, it was between 21°C and 22°C.

It was at first thought that temperature would be related to adhesion by affecting the stability of the adhesive matrix; however, two different temperature ranges for maximum growth were found. Under incandescent light, the maximum adhesion was found between 21°C and 24°C (Figure 11), while under fluorescent light, it was between 20°C and 22°C (Figure 12). If the only effect of temperature was on the stability of the adhesive matrix, these temperature ranges should be the same. What seems to be affecting adhesion here was growth. Maximum growth for incandescent light was 23°C to 28°C, while for fluorescent light, it was 21°C to 22°C. Thus, where growth was best, so was adhesion. If

growth was poor, there would be less material available for synthesis of the adhesive matrix.

The effect of light intensity on adhesion may be the result of a phytochrome response. Phytochrome is found in higher plants and algae. It is a photoreversible pigment existing in two forms, Pr (red absorbing) and Pfr (far-red absorbing). Knowledge of the chemical structure is incomplete, but is known to consist of an open-chain tetrapyrole chromophore attached to a protein. The photoconversion of one form of phytochrome to the other takes several steps and is initiated by specific wavelengths of light. The conversion involves an isomeric change of the chromophore. This photoconversion can be summarized as follows:

$$\text{Pr} \underset{\text{Far-red (730)}}{\overset{\text{Red (660)}}{\rightleftharpoons}} \text{Pfr}$$

Pfr is considered the physiologically active form of phytochrome which can activate or deactivate a particular response. Very little is known about the sequence of events leading from Pfr to a particular response. However, one response of phytochrome is the activation or deactivation of enzymes (14).

The possibility of a phytochrome response affecting adhesion could explain the results concerning the effects of light intensity on adhesion as shown in Figures 12 and 13. Recall from Figure 8 that incandescent light had a high irradiance in the far-red as compared to the red. Thus, the incandescent light would produce a high Pr-to-Prf ratio while the fluorescent light would produce a high Pfr-to-Pr ratio. If the Pfr is the active form and one supposes it is deactivating some system, say enzymes that will degrade the adhesive matrix, then a decrease in Pfr, with an increase in Pr, would activate these enzymes. Once these

enzymes became activated, they would degrade the adhesive matrix causing a loss in adhesion. The incandescent light would produce an increase in Pr and a decrease in Pfr which would activate the degradative enzymes. These enzymes would degrade the adhesive matrix resulting in a loss in adhesion. As the light intensity increased, the decrease in adhesion would become more apparent. This is shown in Figure 12. The fluorescent light would produce an increase in Pfr with an increase in light intensity and this would cause an increase in adhesion as shown in Figure 13. Far-red light will increase Pr and this increase did affect adhesion as shown in Figure 16. An increase in Pr with a decrease in Pfr resulted in a loss of adhesion. Red light on the other hand caused an increase in Pfr with a decrease in Pr. Thus, red light increased adhesion as shown in Figure 16. The effect of alternating exposure to red and far-red is shown in Table 4. This experiment is the classically accepted criterion (14) for establishing phytochrome involvement.

The effect of darkness on the loss of adhesion (Figure 14) can be explained in two ways. First, the adhesive matrix serves a dual role. It affixes the algae to a surface, but also serves as a nutrient reserve which may be reabsorbed by the algae and used to maintain metabolism during the shutdown of photosynthesis. Second, it could be the result of a phytochrome response. As stated before, Pfr reverts to Pr during exposure to far-red light. Pfr also reverts slowly back to Pr under prolonged darkness. Thus, darkness would cause a slow conversion to Pr and an activation of the degradative enzymes.

DMCU was used to find out how darkness causes a loss of adhesion. DCMU being an inhibitor photosystem II would shut down photosynthesis.

If this shutdown of photosynthesis was the cause of the loss of adhesion, then this should be seen in Figure 15, and it is. One might conclude, therefore, that the adhesive matrix was being degraded and reabsorbed by the algae to maintain metabolism. However, the enzymes that do this could be the same enzymes that are induced by the phytochrome response. These enzymes could have more than one way to be activated.

The results obtained when calcium was depleted from the medium suggest the likelihood that calcium ions play an important role in the hardening process that takes place in the adhesive matrix. The role calcium plays is probably the formation of ionic bonds between negatively charged ions. HOEDTA is a specific chelator for calcium. The fact that Na_2EDTA is no more effective in causing loss of adhesion than HOEDTA (Figure 18) suggested that it was only calcium that played an important role in adhesion. When calcium was depleted from the medium, a diffusion of calcium from the adhesive matrix into the medium would take place. This is shown in Figure 17 where it took several days for the calcium concentration to reach zero. This process would be slower than the effects of metal chelators and this is shown in Figure 18. The other components in medium A (Table 5) were ineffective on adhesion as compared to calcium. What effect they did show was probably nutritional. The fact that adhesion was restored when calcium was added back suggests that it is only acting as a strengthening agent by forming ionic bonds in binding side chains.

Christie, Evans and Shaw (5) found that the adhesion of newly-settled zoospores was lost when α-amylase and/or trypsin was added. They also found that, with time, these enzymes lost their effect. From their observations, they felt that the adhesive matrix underwent a

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chemical change or a hardening after the adhesive material was secreted. When I added α-amylase and trypsin to two-week-old cultures, there was only a slight effect on adhesion (Figure 19). This effect was similar to that of Christie, Evans and Shaw. I feel that calcium plays the primary role in this hardening process. The slight effect of these enzymes also suggests that the adhesive matrix is composed of protein and carbohydrate.

To summarize, the adhesive material of Enteromorpha was composed of protein and carbohydrate with calcium interacting in a way to strengthen the matrix. The adhesive matrix appeared to remain stable as the algae aged. There was a phytochrome response that could cause the loss of adhesion or maintain it. The adhesive material might also play a role in supplying needed compounds during periods of nutritional need. The cells of the holdfast structure might secrete a toxic substance that inhibits growth of adjacent structures. These findings of various factors that affect adhesion might possibly be exploited in trying to inhibit the attachment of Enteromorpha to the hulls of ships.

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